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Cover photo: Adult female *Hypochilus pococki* (Hypochilidae), probably gravid, on the side of a large riverside boulder on the Chattooga River, North Carolina (see page 167). Photo by Marshal Hedin.

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A new highly troglomorphic species of *Eukoenenia* (Palpigradi: Eukoeneniidae) from tropical Brazil

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Abstract. A third troglobitic species of Brazil is described from three specimens collected within Gruta da Morena, located in the municipal district of Cordisburgo, Minas Gerais. *Eukoenenia sagarana* new species (Palpigradi: Eukoeneniidae) is highly adapted to subterranean environments, corresponding to the most troglomorphic Palpigradi species described to date. This new species is very close to *E. maquinensis* Souza & Ferreira 2010, a troglobitic species recently described from Gruta de Maquiné, located in the same municipal district. The differences and similarities between these two species and between *E. sagarana* and other species of *Eukoenenia* are presented.

Keywords: *Eukoenenia sagarana*, Neotropics, taxonomy, morphology

The order Palpigradi Thorell 1888 is considered one of the lesser-known groups within the Arachnida (Pepato et al. 2010). Fortunately, in recent years studies on this order have become more frequent (Barranco & Harvey 2008; Christian 2009; Christian et al. 2010; Souza & Ferreira 2010, 2011a, 2011b). However, data on Neotropical palpigrades are still very limited, and even more restricted for troglobitic Neotropical species, of which only three species have been described: *Eukoenenia orghidani* Condé & Juberthie 1981 from Cuba, *E. maquinensis* Souza & Ferreira 2010 from Brazil, and *E. spelunca* Souza & Ferreira 2010, also from Brazil.

The most distinetive troglomorphisms are found in species of the genus *Eukoenenia* Börner 1901. The morphological adaptations to the cave environment in this group consist mainly of increases in body size, elongation of the appendages (that also become thinner) and the increase in the number of sensory receptors, which correspond with the increase in elements that constitute the lateral organs (Condé 1988, 1998). According to Condé (1998), the length of basitarsus IV is the most reliable parameter to quantify the elongation of the appendages because, related to the length of the tibia, it provides an index of adaptation to the cave environment. In edaphic species, the tibia is longer than the basitarsus, forming a basitarsus:tibia ratio of less than one, while in troglobitic species the ratio will be close to, equal to, or higher than one. The value of the ratio among the lengths of the propeltidium and the basitarsus IV is also used to indicate possible levels of troglomorphism, and in edaphic species the value is approximately between three and four while in the cave species, it is less than two (Condé 1998).

The setae of the propeltidium, represented by ten pairs in most species, have a tendency to become smaller and sometimes less numerous in troglobites. A decrease in the number and size of the setae also occurs on the metapeltidium, with two pairs or one occurring instead of three (Condé 1992, 1998).

These troglomorphisms vary from each other in an independent way, according to the perceived evolutionary stage of the species (Condé 1998). Thus, for some species, certain structures will be more troglomorphic, while in others, more accentuated troglomorphisms can occur in different structures. Each species may acquire a series of unique characteristics due to its particular evolution in the subterranean environment, which makes it difficult to compare the species' degree of troglomorphism effectively. As a result, it is necessary for the group of characteristics to be analyzed as a whole and not each one in isolation.

In this work a new Brazilian species of *Eukoenenia* is described, which was collected in Gruta da Morena, near Cordisburgo, Minas Gerais. This new species is the second species of troglobitic palpigrade described from this municipal district and the third described from Brazil. Furthermore, it exhibits several notable troglomorphisms, and is considered here to be the most troglomorphic species of Palpigradi described to date.

METHODS

The specimens were examined by clearing in Nesbit's solution and mounting them in Hoyer's medium on 7.6 × 2.5-cm glass slides using the standard procedures developed for mites (Krantz & Walter 2009). All measurements are presented in micrometers (μm) and were taken using an ocular micrometer with a phase contrast microscope. We measured body length from the apex of the propeltidium to the posterior margin of the opisthosoma. The areoles in some drawings represent the insertions of setae.

Setal nomenclature and abbreviations follow Barranco & Mayoral (2007): L, total body length (without flagellum); B, dorsal shield length; P, pedipalpus; I and IV, legs I and IV; ti, tibia; bta1, basitarsus 1; bta2, basitarsus 2; bta3, basitarsus 3; bta4, basitarsus 4; ta1, tarsus 1; ta2, tarsus 2; ta3, tarsus 3; a, width of basitarsus IV at level of seta r; er, distance between base of basitarsus IV and insertion of seta r; grt, tergal seta length; gla, lateral seta length; r, stiff seta length; t/r, ratio between length of basitarsus IV and stiff seta length; t/er, ratio between basitarsus IV length and distance to insertion of stiff seta; gla/grt, ratio between lengths of lateral and tergal setae; B/bta, ratio between lengths of prosomal shield and basitarsus IV; bta/ti, ratio between lengths of basitarsus IV and tibia IV.

The specimens are lodged in the Coleção de Invertebrados Subterrâneos de Lavras, Departamento de Biologia, Universidade Federal de Lavras, Lavras, Minas Gerais (ISLA).

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TAXONOMY

Family Eukoeneniidae Petrunkevitch 1955
Genus *Eukoenenia* Börner 1901

Koenenia Grassi & Calandruccio 1885:165 [junior primary homonym of *Koenenia* Beushausen 1884 (Mollusca: Bivalvia)].

Koenenia (*Eukoenenia*) Börner 1901:551.

Type species.—*Koenenia mirabilis* Grassi & Calandruccio 1885, by monotypy.

Eukoenenia sagarana new species
Figs. 1–24

Material examined.—Male holotype, Brazil, Minas Gerais, Gruta da Morena cave (UTM 23 569484–7880316), Cordisburgo, 29 September 2010, R.L. Ferreira (ISLA 1409). Paratypes: 1 female, same data as holotype except 16 December 2009 (ISLA 1410); 1 juvenile, same data as holotype except 1 April 2010 (ISLA 1411).

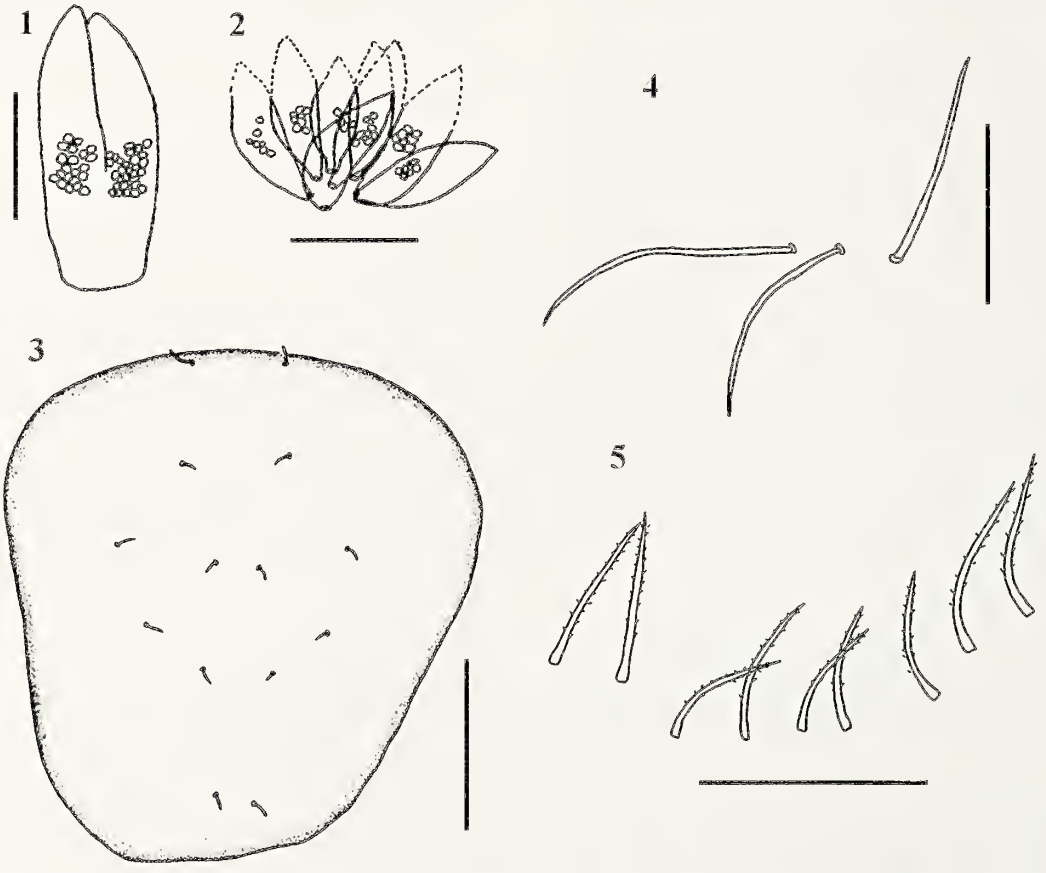
Diagnosis.—*Eukoenenia sagarana* differs from all other species of the genus by the following combination of characters: presence of 8–9 blades on prosomal lateral organs; propeltidium with 7 + 7 setae; six setae on basitarsus IV with a single proximal sternal seta; opisthosomal sternites IV–VI with 14, 14, and 13 setae, respectively, in the male and 15, 13 and 10 setae, respectively, in the female; and the singular shape of the spermatheca in the female genitalia.

Description of the adult stage.—Prosoma: frontal organ with two branches, blunt apically, each 5 times longer than wide (37.5 µm/7.5 µm) (Fig. 1). Lateral organ with eight pointed blades (nine blades in the male on the left side), each 3.25 times longer than wide (32.5 µm/10 µm) (Fig. 2). Propeltidium with 7 + 7 short setae, first pair on either side of frontal organ longer than others (Fig. 3). Metapeltidium with t_1, t_2, t_3 (157.5 µm, 125 µm and 117.5 µm) (Fig. 4). Deutotritosternum with nine setae in U-shaped arrangement (eight setae in the male) (Fig. 5).

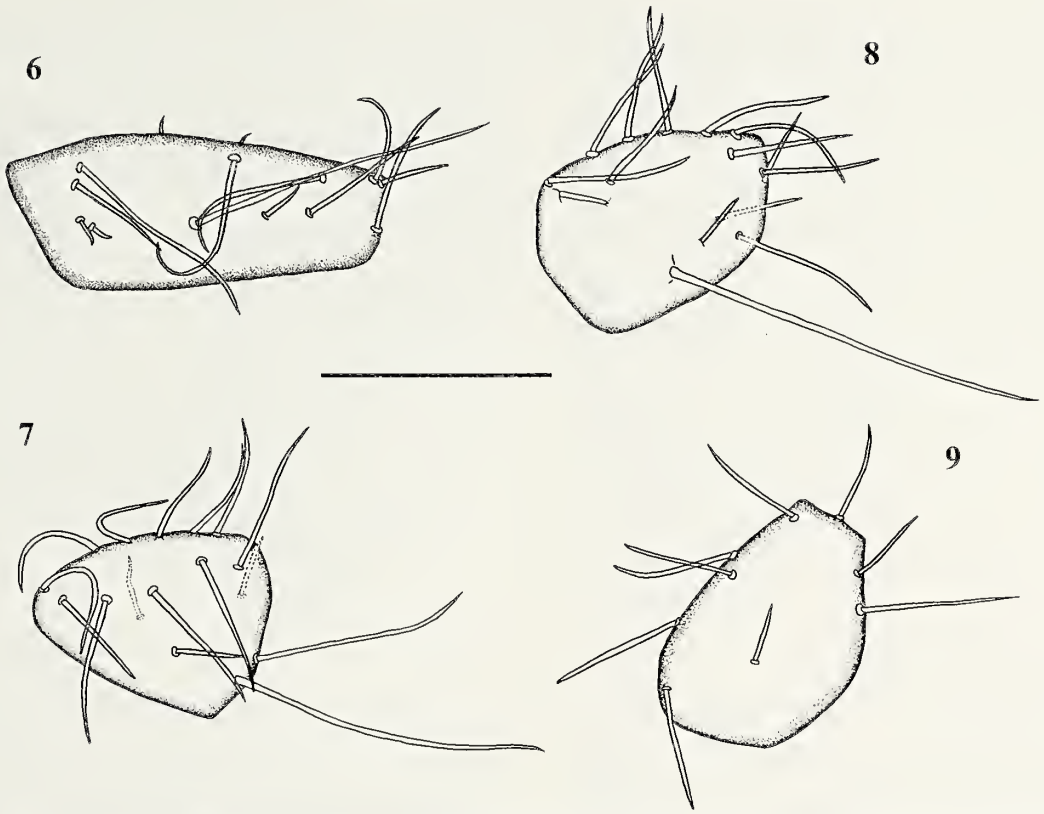
Coxal chaetotaxy: coxa I with 14 setae, coxa II with 6 thick and 10 normal setae, coxa III with 7 thick and 8 normal setae (5 thick and 9 normal setae in the male) and coxa IV with 2 thick and 7 normal setae (8 normal setae in the male) (Figs. 6–9).

Chelicerae with 9 teeth on each finger; 1 dorsal seta and 1 ventral seta inserted near the third segment, 1 seta inserted near the row of teeth of the second segment and a row with 3 setae inserted in a middle region (between the dorsal and ventral seta) (Fig. 10).

Basitarsus 3 of leg I slender, 4.7 times longer than wide, with 3 setae (grt 155 µm; r 132.5 µm). Seta r shorter than segment (152.5 µm/132.5 µm, t/r = 1.1), inserted in proximal half and surpassing hind edge (160 µm/45 µm, s/er = 3.5) (Fig. 11). Basitarsus of leg IV long, 11 times longer than wide, with 6 setae (2 esd, esp, gla, grt and r) (Fig. 12), bta/ti 1.02. Stiff seta r 2.7 times shorter than tergal edge of article



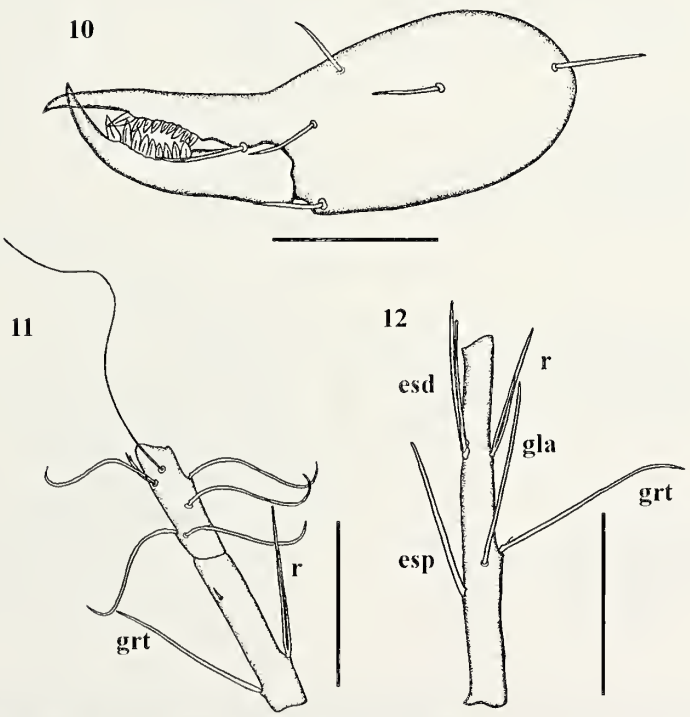
Figures 1–5.—*Eukoenenia sagarana* new species, female: 1. Frontal organ, dorsal view; 2. Lateral organ, dorsal view; 3. Propeltidial chaetotaxy; 4. Metapeltidial setae; 5. Deutotritosternal setae. Scale bars 20 µm (Fig. 1), 20 µm (Fig. 2), 150 µm (Fig. 3), 100 µm (Fig. 4), 60 µm (Fig. 5).



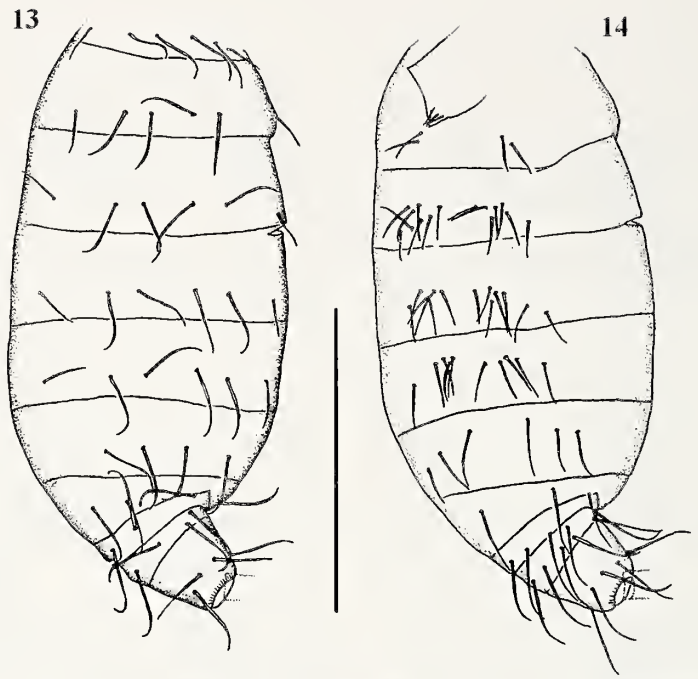
Figures 6–9.—*Eukoenenia sagarana* new species, female: 6. Coxa I; 7. Coxa II; 8. Coxa III; 9. Coxa IV. Scale bar 150 μm.

(302.5 μm/112 μm, t/r = 2.7) and inserted in distal third (302.5μm/187.5 μm, t/er = 1.61). Seta esp proximally inserted, followed by gla and grt, more or less at the same level, all of them in proximal half.

Opisthosoma: female: tergites II–VI with 3 + 3 setae each, 2 pairs of tergal setae (t_1, t_3) between both slender setae (s) (Fig. 13). Sternite III with 2 + 2 setae. Sternite IV with 15 setae (13 thickened setae between both slender setae); sternite V



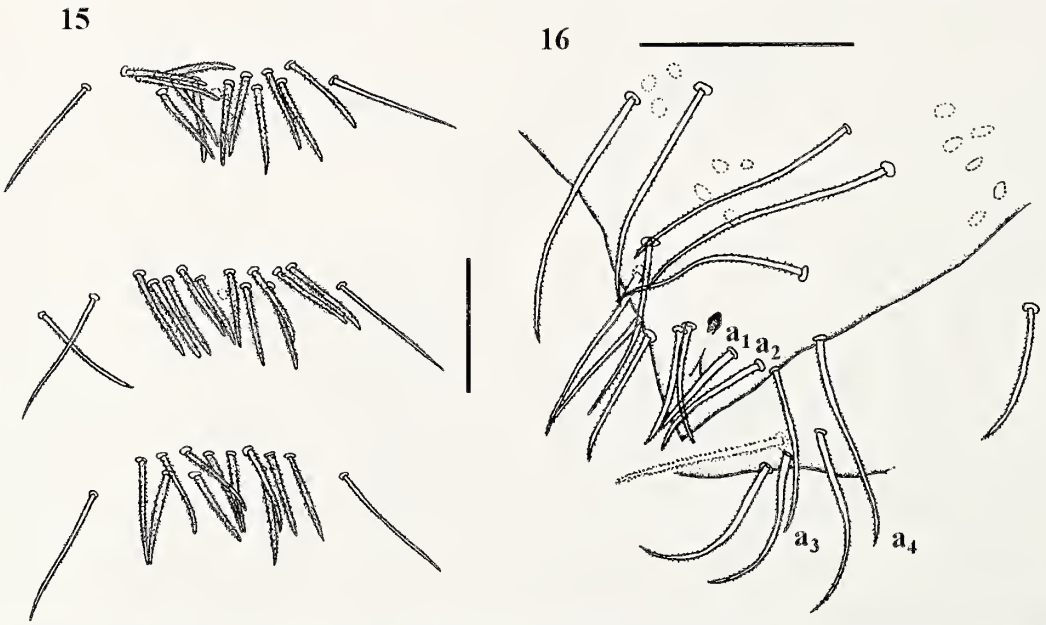
Figures 10–12.—*Eukoenenia sagarana* new species, female: 10. Chelicera; 11. Basitarsus 3–4 of leg I; 12. Basitarsus IV. Scale bars 150 μm.



Figures 13,14.—*Eukoenenia sagarana* new species, female: 13. Opisthosoma, dorsal view; 14. Opisthosoma, ventral view. Scale bar 600 μm.

with 13 setae (11 thickened setae between both slender setae); sternite VI with 10 setae (8 thickened setae between both slender setae). Sternites IV–VI each with one glandular pore on the median line. Segments VII–XI each with 8, 10, 9, 9 and 8 setae, respectively (Fig. 14). Male: tergites II–VI and sternite III as in female. Sternite IV with 14 setae (12 thickened setae between both slender setae); sternite V with 14 setae (11 thickened setae between two slender setae on one side and one on the other); sternite VI with 13 setae (11 thickened setae between both slender setae). Setae of the a-groups considerably thicker and subcylindrical than that found in the female. Sternites IV–V each with two glandular pores on the median line (Fig. 15). Segments VII–XI eaeh with 8, 9, 8, 8 and 8 setae, respectively.

Female genitalia: first lobe with 11 + 12 setae in 5 transverse rows, 4 sternal 2 + 2, 2 + 3 (asymmetry caused by disloeations due to the lack of regular and/or the presence of additional setae), 2 + 2, 1 + 1 and distal 4 + 4, of which a_1, a_2, a_3, a_4 measure 30–32.5 μm, 35–40 μm, 42.5–45 μm and 57.5 μm, respectively. Second lobe with 3 + 3 setae (x, y, z) (second lobe is curved doubled bent), measuring 40 μm, 55 μm and 42.5 μm, respeetively; 13 glandular orifices (Fig. 16). Spermatheca illustrated in Fig. 16.



Figures 15,16.—*Eukoenenia sagarana* new species: 15. Opisthosomal sternites IV–VI of male; 16. Female genitalia. Scale bars 100 μm (Fig. 15), 60 μm (Fig. 16).

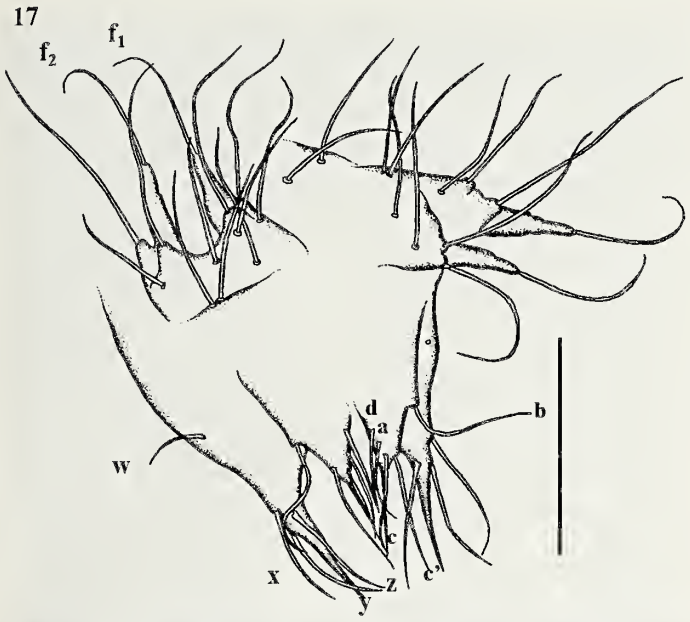
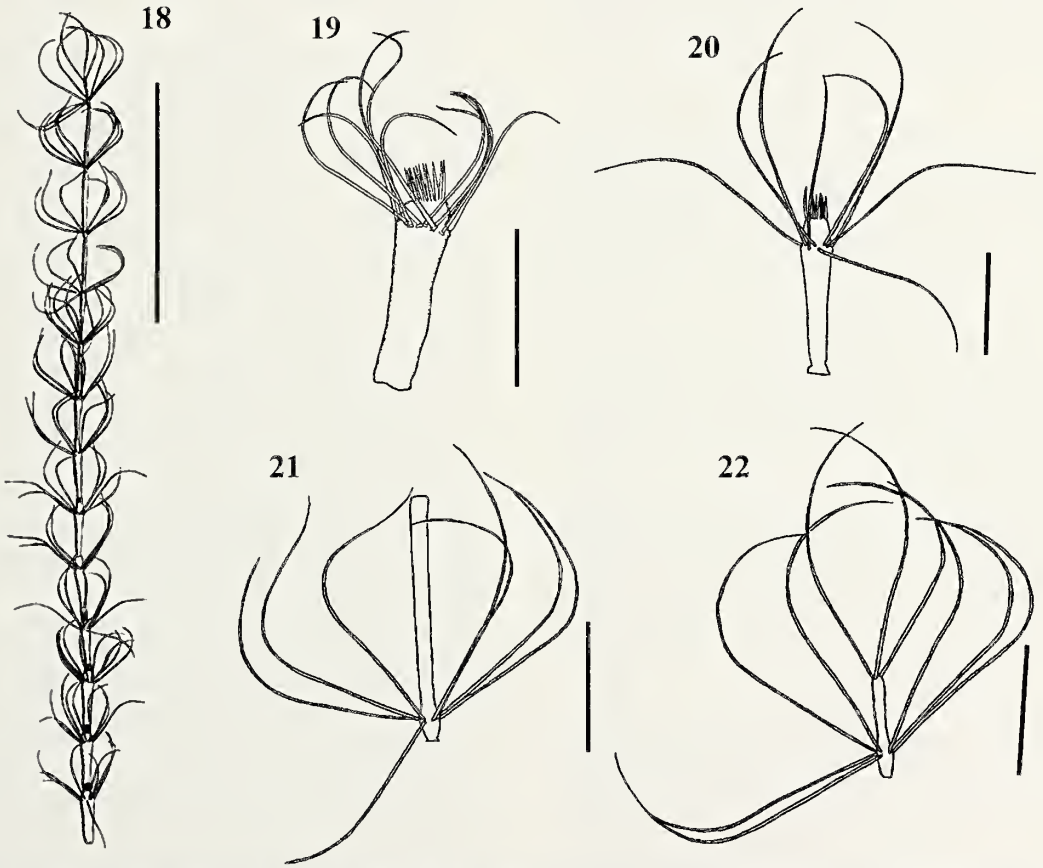


Figure 17.—*Eukoenenia sagarana* new species, holotype: male genitalia. Scale bar 150 μm.

Male genitalia: first lobe appears as two elements, wider than long, with 13 + 13 setae (including 2+2 fusules in the distal margin); f_1 = 150–152.5 μm; f_2 = 140–145 μm. Second lobe subtriangular, with a simple and sharp apex (without bifurcation), with 5 + 5 setae (a, b, c, c', d). Third lobe also in a subtriangular form, well developed, with 4 + 4 setae (w, x, y, z), with a large, sharp and simple acute apical region (Fig. 17).

Flagellum: longer than body length, with 14 long, slender segments (Fig. 18). Segments 1, 3, 5, 7 and 9 with an apical crown of thorns. Segments 1–9 with 12, 9, 11, 11, 8, 10, 8, 10 and 8 long setae, respectively, inserted in the distal half (Figs. 19–20). Tenth segment with 8 long setae inserted in the distal half, but closer to the middle of the segment. Eleventh segment with 8 long setae in proximal half (Fig. 21). Twelfth and thirteenth segments with 8 long setae inserted in proximal third. Last segment with 7 long setae inserted in proximal third of segment and 3 setae inserted apically (Fig. 22).

Description of juvenile female (larve B *sensu* Condé 1996): Frontal organ with two branches, each 5 times longer than wide (25 μm/5 μm). Lateral organ with seven blades, each 4.25 times longer than wide (42.5 μm/10 μm). Deutotritosternum with 6 setae. Fingers of chelicera with 8 teeth. Chaetotaxy of propeltidium and metapeltidium complete. Coxal chaetotaxy: coxa I with 13 setae, coxa II with 4 thick and 10 normal setae, coxa III with 4 thick and 10 normal setae and coxa IV with 1 thick and 8 normal setae. Trichobothria and forked setae as in adult. IV bta and opisthosomal tergites II–VI similar to the adult. Sternites IV–VI with 13, 14 and 14 setae, respectively. Segments VII–XI with 10, 8, 8, 8 and 8 setae. Primordia of genital lobes developed on segments II and III. Segment II with 6 + 6 setae and segment III with 1 + 1 setae (Fig. 23). The



Figures 18–22.—*Eukoenenia sagarana* new species, female: 18. Flagellum (the first segment is lacking); 19. First flagellar segment; 20. Fifth flagellar segment; 21. Eleventh flagellar segment; 22. Last flagellar segment. Scale bar 1300 μm (Fig. 18), 200 μm (Figs. 19–22).

Figure 23.—*Eukoenenia sagarana* new species, juvenile female: primordia of genital lobes. Scale bar 60 μm.

habitus of the immature specimen (without the flagellum) is shown in Fig. 24.

Morphometric data are given in Table 1.

Etymology.—The specific name refers to *Sagarana*, a book of stories by the famous Brazilian writer João Guimarães Rosa, born in Cordisburgo. *Sagarana* is formed by a hybridism: "*saga,*" radical of Germanic origin that means "legend"; and "*rana*" word of Tupi origin (Brazilian Indian language), that means "that which expresses similarity." Therefore, *Sagarana* means "close to a legend." It is to be treated as a noun in apposition.

DISCUSSION

Sexual dimorphism in the Palpigradi is quite conspicuous in *Koeneniodes* Silvestri 1913 and *Prokoenenia* Börner 1901 and more discrete in the other genera. It arises mainly from the glandular complexes and setae located on sternites IV–VI (Condé 1991). In *E. sagarana,* differences can be observed in the number and form of the thickened setae, since the male has more numerous and considerably thicker and more cylindrical setae than the female. Similar differences can be observed in other species of the genus *Eukoenenia* such as *E. janetscheki* Condé 1993, *E. tetraplumata* Moreno 2007 and *E. maroccana* Barranco & Mayoral 2007 (Condé 1993, 1997; Moreno 2006; Barranco & Mayoral 2007).

Other species of the genus *Eukoenenia* also have numerous setae a on the opisthosomal sternites: *E. pretneri* Condé 1977 (5a + 5a on sternite IV, 6a + 6a on sternite V and 1a + 1a on sternite IV), *E. bouilloni* Condé 1980 (sternites IV–VI with 10, 9 and 7 thick setae, respectively) and *E. bonadonai* Condé 1979 (sternites IV–VI with two regions of thick setae, approximately symmetrical and contiguous including 11 + 12 bristles on IV, 14 + 13 on V and 12 + 13 on VI). In these species, however, it is not known if there is sexual dimorphism in the number of thick setae on the opisthosoma, as only the males are known. The female of *E. maquinensis* also has numerous setae on sternites IV–VI (14, 13 and 11 respectively), having little differentiation between thickened setae (a) and normal setae (s) located in these segments (Souza and Ferreira 2010), which also occurs in the female of *E. sagarana.*

Figure 24.—*Eukoenenia sagarana* new species, juvenile female: habitus, dorsal view.

The same asymmetry present in the genitalia of the female holotype of *E. maquinensis* (12 + 11 setae on the first lobe) is also observed in *E. sagarana.* The male genitalia have a unique form and chaetotaxy, with relatively long and prominent fusules.

Eukoenenia sagarana is a troglobitic species very similar to *E. maquinensis,* the first described troglobitic species from South America (Souza and Ferreira 2010). The first species occurs in the Gruta da Morena cave and the second in the Gruta de Maquiné, both located in the municipal district of Cordisburgo (Minas Gerais), and only about 5 km from each other. However, a stream crosses between the two caves, which may impede sub-surface migration of organisms between these systems. These two species share many important taxonomic characteristics, mainly being distinguished by the number of elements that form the lateral organs, the disposition of the setae in the chelicerae, the number of setae of the abdominal sternites IV–VI, and the form of the spermatheca.

Both show quite accentuated troglomorphisms, as females of both species have a bta VI/Ti ratio equal to 1.07 (1.02 in the male and 0.94 in the juvenile of *E. sagarana*). However, *E. sagarana* also has a higher degree of adaptation to the cave environment when compared not only to *E. maquinensis,* but also to the highly troglomorphic Greek species *E. naxos* Condé 1989, for having a B/btaIV ratio of 1.43, a value even lower than that presented by these two species (1.58–1.7 in *E. maquinensis* and 1.71 in *E. naxos*) (Condé 1989; Souza & Ferreira 2010). Also, the new species has an extremely

Table 1.—Measurements (μm) of selected body parts of the specimens of *Eukoenenia sagarana* new species.

Body part	Male (holotype)	Female (paratype)	Juvenile female
L	2020	1900	1595
B	435	420	352.5
Pti	262.5	242.5	190
Pbta1	102.5	87.5	72.5
Pbta2	117.5	112.5	85
Pta1	57.5	55	45
Pta2	82.5	72.5	60
Pta3	100	92.5	77.5
Iti	-	327.5	-
Ibta1+2	285	252.5	-
Ibta3	152.5	147.5	105
Ibta4	130	120	90
Ita1	72.5	72.5	55
Ita2	67.5	67.5	62.5
Ita3	222.5	232.5	190
IVti	295	272.5	222.5
IVbta	302	292.5	210
IVta1	107.5	97.5	80
IVta2	137.5	125	110
A	27.5	25	25
Er	187.5	197.5	145
Grt	192.5	160	127.5
Gla	155	150	105
R	112	102.5	77.5
tlr	2.7	2.85	2.7
tler	1.61	1.48	1.44
gla/grt	0.8	0.93	0.82
B/btaIV	1.43	1.43	1.67
btaIV/tiIV	1.02	1.07	0.94
FI	237.5	220	-
FII	280	-	-
FIII	305	-	-
FIV	330	-	-
FV	280	-	-
FVI	345	-	-
FVII	282.5	-	-
FVIII	345	-	-
FIX	272.5	-	-
FX	370	-	-
FXI	430	-	-
FXII	360	-	-
FXIII	355	-	-
FXIV	150	-	-

elongated basitarsus IV, being 11.7 times longer than wide in the female. This value is 8.8 in *E. maquinensis* and 10.22 in *E. naxos*. The number of elements that constitute the lateral organ in *E. sagarana* (8–9) is larger than that observed in those two species (6 and 5 respectively). The presence of 8 elements forming the lateral organ is an uncommon characteristic (as shown in the female of *E. sagarana*), being shared with only five species of *Eukoenenia*: *E. grafittii* Condé & Heurtault 1993 (8), *E. lyrifer* Condé 1992 (8), *E. patrizii* (Condé 1956) (8–10), *E. draco* (Peyerimhoff 1906) (8) and *E. hispanica* (Peyerimhoff 1908) (8). Furthermore, it is important to point out that Souza & Ferreira (2010) had considered *E. maquinensis* as the species with the longest known flagellum (3.865 mm). *Eukoenenia sagarana*, in turn, has the longest flagellum described to date, with a length of 4.3 mm.

When comparing the many characteristics shared by *E. sagarana* and *E. maquinensis*, it is plausible to assume that these two species originated from a common ancestor. Probably this one ancestor took shelter in hypogean habitats during drastic climatic changes, withstanding isolation and forming these two species that remain restricted to the Gruta da Morena and Gruta de Maquiné caves. Due to the absence of efficient external inventories, it is difficult to determine if this ancestral surface lineage still exists or is locally extinct. What can be affirmed is that this isolation occurred a long time ago, keeping in mind the high degree of adaptation to the subterranean environment presented by these species. In some caves of the area (including the Gruta da Morena), as well as in some epigean habitats, edaphomorphic Palpigradi have been found, probably belonging to the species *E. florenciae* (Rucker 1903). However, there is no indication that *E. maquinensis* and *E. sagarana* have any phylogenetic relationship with this species, since they do not share any morphological characteristics. Therefore, it is possible that *E. florenciae* colonized the area after the speciation event that gave rise to the troglobitic species.

Eukoenenia naxos was considered by Condé (1998) to be the species that reached the highest degree of subterranean evolution for a range of characteristics, except for the number of lateral organs. Among the various troglomorphisms shown by this species, the substitution of the tergal seta of basitarsus IV by a trichobothrium can be mentioned and the largest bta IV/ti ratio observed for a type of Palpigradi. However, the great elongation of the appendages evidenced by the B/bta IV ratio and by the ratio between the length and width of bta IV, the presence of 8–9 blades forming the lateral organs and the extreme elongation of the flagellomeres suggests *E. sagarana* has more troglomorphic characteristics than *E. naxos*. Therefore, this new species can be considered the palpigrade species most adapted to the subterranean environment described to date. This fact reinforces the idea presented by Souza & Ferreira (2010) that the effects of climatic changes in the Neotropical region were similar to the temperate region on some groups, such as the palpigrades.

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Three new spider species of Anapidae (Araneae) from China

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Abstract. Three new species of the family Anapidae are reported from caves and tropical rainforest of southern China: *Gaiziapis encmensis*, *Minanapis menghunensis* and *Sinanapis longituba*. The genus *Minanapis* is recorded for the first time from China.

Keywords: Anapid, tropical rainforest, cave spiders, taxa

The family Anapidae was erected by Simon (1895). Anapid members are small (usually less than 3 mm in body length), three-clawed, ecribellate, haplogyne, cryptozoic spiders with six or eight eyes situated on an elevated ocular region. They usually live in leaf litter and moss on the rainforest floor and build orb webs with a diameter of less than 3 cm (Murphy et al. 2000). Some also inhabit caves. This family was redefined by Platnick and Shadab (1978, 1979). Platnick and Forster (1989) supposed that the labral spur and the glandular openings at anterolateral corners of the carapace were two synapomorphies for the family Anapidae.

The family Anapidae includes 38 genera and 150 species (Platnick 2011), distributed in tropical and southern temperate regions, such as Central and South America, Australia and Southeast Asia. The anapid species from China are insufficiently studied. Brignoli (1981) first reported an anapid species, *Pseudanapis serica* Brignoli 1981 from Hong Kong. Since then four species, *Comaroma tongjunca* Zhang & Chen 1994 (from Zhejiang), *Sinanapis crassitarsa* Wunderlich & Song 1994 (from Yunnan), *Emielkenie acaroides* Ono 2006 (from Taiwan) and *Gaiziapis zhizhnba* Miller, Griswold & Yin 2009 (from Yunnan) have been reported from China. The present paper deals with three new Chinese species of the family Anapidae based on material collected in Guangxi, Yunnan and Hainan.

METHODS

Specimens were examined using a Leica M250 C stereomicroscope. Further details were studied under an Olympus BX51 compound microscope. All drawings were made using a drawing apparatus attached to an Olympus BX51 compound microscope, and then inked on ink jet plotter paper. Male palpi and female genitalia were examined and illustrated after they were dissected from the spiders' bodies. Vulvae of females were removed and treated in lactic acid before illustration. Male palpi and female vulvae were illustrated by incident light against a white baekground after being embedded in Hoyer's Solution. Type specimens are deposited in the Institute of Zoology, Chinese Academy of Sciences in Beijing (IZCAS).

All measurements were made under a Leica M250 C stereomicroscope and are given in millimeters. Leg measurements are shown as total length (femur, patella, tibia, metatarsus, and tarsus). Abbreviations used in figures are as follows: AA = apical apophysis; BA = basal apophysis; CD = copulatory duct; Cm = cymbium; CO = copulatory opening; Co = conductor; DS = dorsal scutum; EF = epigynal furrow; Em = embolus; FA = femoral apophysis; FD = fertilization duct; Fe = femur; LS = labral spur; MA = median apophysis; PA = patellar apophysis; Pa = patella; POG = postgenital plate; S = spermatheca; Ti = tibia; Tu = tegulum and VS = ventral scutum.

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TAXONOMY

Family Anapidae Simon 1895

Genus *Gaiziapis* Miller, Griswold & Yin 2009

Gaiziapis encmensis **new species**

Figs. 1–3, 10

Type material.—Holotype ♂ (IZCAS), CHINA: *Gnangxi*: Nandan County, Chengguan Town, Encun Village, Liangfeng Cave (25°04′N, 107°38′E), elevation 598 m, 4 March 2007, J. Liu and Y. Lin. Paratypes: 25 ♂, 30 ♀ (IZCAS), same as holotype.

Etymology.—The specific name refers to the type locality; adjective.

Diagnosis.—The new species is similar to *G. zhizhnba* in sharing the following characters: a deep anteromedian invagination on the dorsal scutum (Fig. 1B), lack of a prolateral apophysis on the palpal bulb and having a much more complicated pedipalp with more membranes and sclerites (Fig. 2B) in the male, the absence of pedipalp in the female and a round, rather than triangular, abdomen from dorsal view (Figs. 1B, D). It can be distinguished from *G. zhizhnba* by a triangular median apophysis (Figs. 1I, 2B) and an apical apophysis with coarse veins on the male palpal bulb (Figs. 1H, 2A), absence of tufty denticles on the tegulum (present in *G. zhizhnba*: Figs. 60A, 61A,C) in the male, and by the club-shaped and translucent spermathecae, the wider and weakly sclerotized copulatory ducts, and the distinctly smaller book lung covers in the female (Figs. 3A, B).

Description.—*Male (Holotype):* Total length 1.20. Carapace 0.54 long, 0.44 wide, 0.60 high. Clypeus 0.22 high. Sternum 0.34 long, 0.30 wide. Abdomen 0.74 long, 0.70 wide, 0.96 high. Carapace brown, smooth, without any modification, anterolateral depressions present, small; cephalic pars risen, covered with short setae. Eight eyes in four diads, round, white; anterior median eyes smallest, other eyes subequal in size, lateral eyes adjacent. From dorsal view, both eye rows straight, same width. Thoracic groove distinct, thoracic pars granulated. Chelicerae with long setae anteriorly, fang furrow

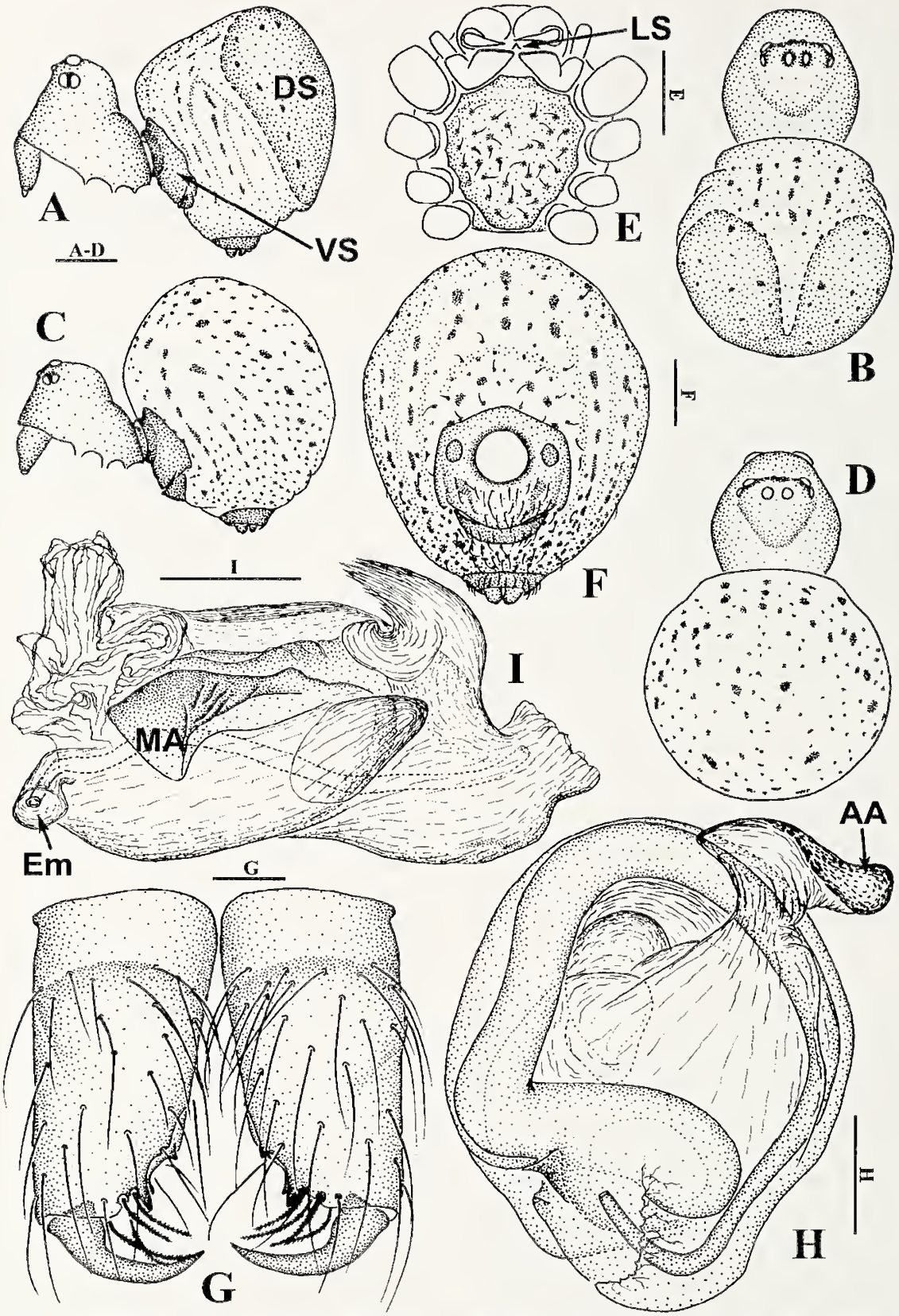

Figure 1.—*Gaiziapis encunensis* **new species**, holotype male and paratype female from Guangxi. A. Male body, lateral; B. Same, dorsal; C. Female body, lateral; D. Same, dorsal; E. Male sternum and coxa, ventral; F. Female abdomen, ventral; G. Male chelicerae, anterior; H. Tegulum, ventral; I. Embolic division, ventral. Scales: A–F = 0.20; G–I = 0.05.

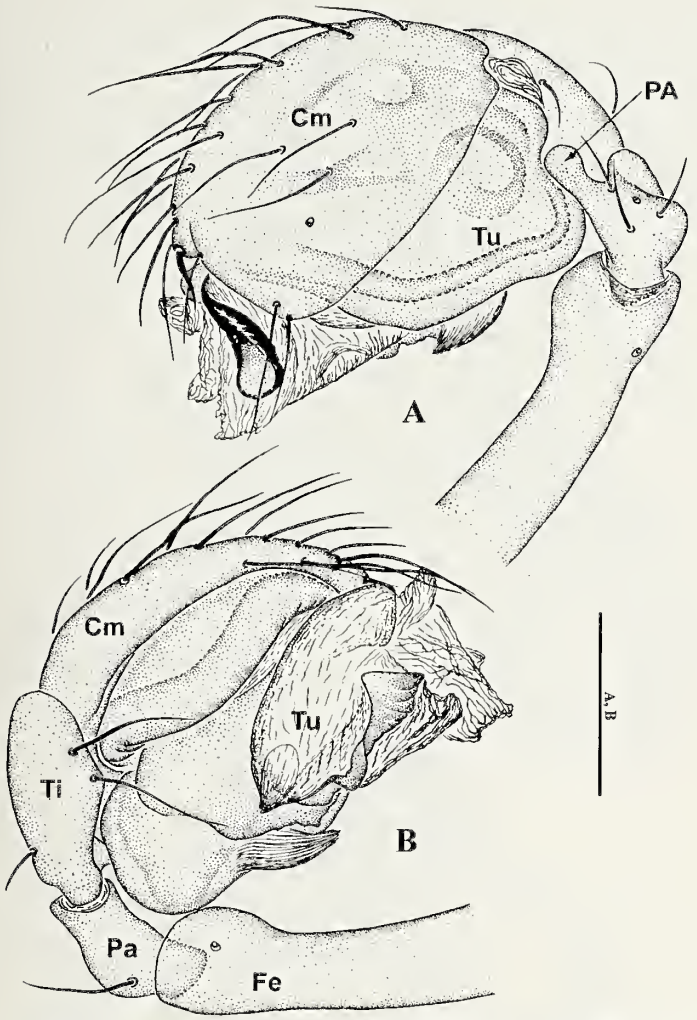

Figure 2.—*Gaiziapis encunensis* **new species**, holotype male from Guangxi. A. Left palp, retrolateral; B. Same, prolateral. Scale = 0.10.

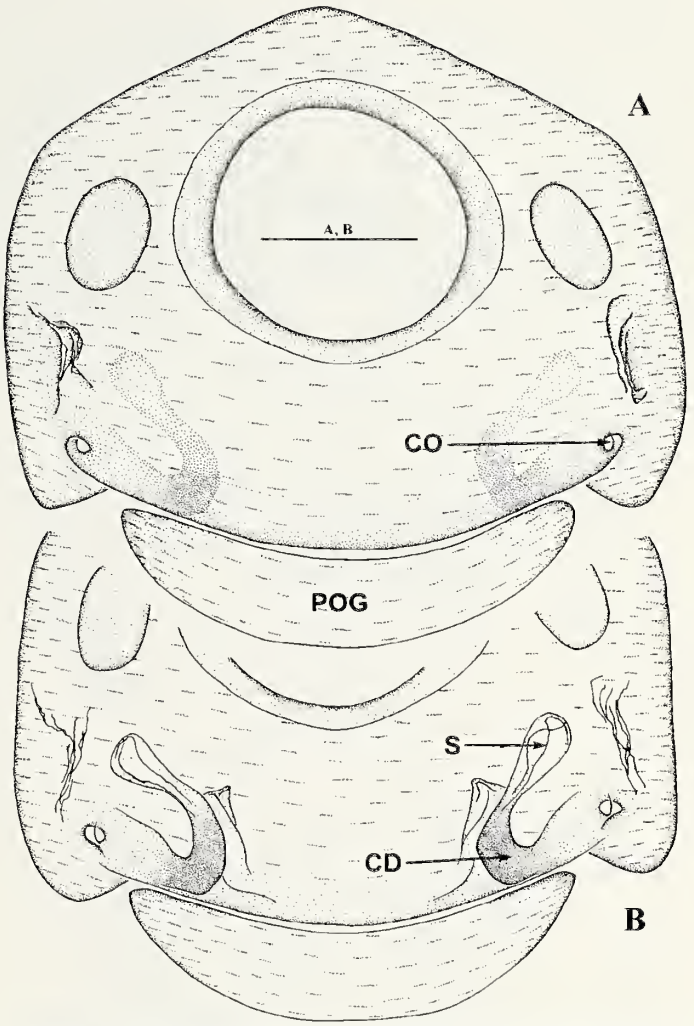

Figure 3.—*Gaiziapis encunensis* **new species**, paratype female from Guangxi. A. Epigynum, ventral; B. Same, dorsal. Scale = 0.10.

with one promarginal and two retromarginal teeth; two plumose setae at promargin and retromargin separately. Labral spur present, small triangular, not furcated. Sternum brown, covered with tiny knurls and short setae. Legs yellow-brown, distal patella and middle tibia with one dorsal spine separately, tibiae with three trichobothria. Leg measurements: I 2.28 (0.78, 0.20, 0.60, 0.26, 0.44); II 1.84 (0.58, 0.16, 0.48, 0.22, 0.40); III 1.44 (0.44, 0.14, 0.34, 0.20, 0.32); IV 1.70 (0.56, 0.14, 0.44, 0.20, 0.36). Leg formula: I-II-IV-III. Abdomen round from dorsal view, covered with sclerotized spots and short setae. Dorsal scutum posterior, split at midline. Spinneret area with a sclerotized annular plate. Palpal patella with a distal retrolateral apophysis. Embolus short, embolic division with a triangular median apophysis and rugose apical lobes. Tegulum wide, with a sclerotized apical apophysis which is modified by granules (Fig. 1H). Ejaculatory duct arising on prolateral side of bulbous base.

Female: (one of the paratypes). Total length 1.12. Carapace 0.48 long, 0.40 wide, 0.44 high. Clypeus 0.20 high. Sternum 0.30 long, 0.28 wide. Abdomen 0.70 long, 0.78 wide, 0.90 high. Same coloration and modification on carapace as in male. Cephalic area slightly lower than in male. Anterior eye row narrower than posterior eye row. Pedipalp absent. Leg measurements: I 1.94 (0.66, 0.16, 0.52, 0.22, 0.38); II 1.66 (0.52, 0.16, 0.42, 0.20, 0.36); III 1.32 (0.40, 0.14, 0.30, 0.18, 0.30); IV 1.60 (0.52, 0.14, 0.42, 0.20, 0.18). Leg formula: I-II-IV-III. Abdomen without dorsal scutum, covered with sclerotized spots dorsally and laterally. Ventral scutum modified by wrinkles at lateral margins. Book lung covers small, ovate. Spinneret area with an annular sclerotized plate. Spermathecae relatively small, clubbed, translucent; copulatory duct curved in the middle, its proximal end fused to the retromargin of pulmonary plate; copulatory duct opens small, distinct.

Distribution.—Known only from the type locality (Fig. 10).

Genus *Minanapis* Platnick & Forster 1989

Minanapis menglunensis **new species**

Figs. 4–6, 10

Type material.—Holotype ♂ (IZCAS), CHINA: *Yunnan*: Mengla County, Menglun Town, rubber plantation (21°55′N, 101°17′E), elevation 556 m, 10–20 June 2007, G. Zheng. Paratypes: 7 ♂, 6 ♀ (IZCAS), the same data as for holotype.

Etymology.—The specific name refers to the type loeality; adjective.

Diagnosis.—This new species is similar to these members of *Minanapis* in the absence of depressions on the anterolateral

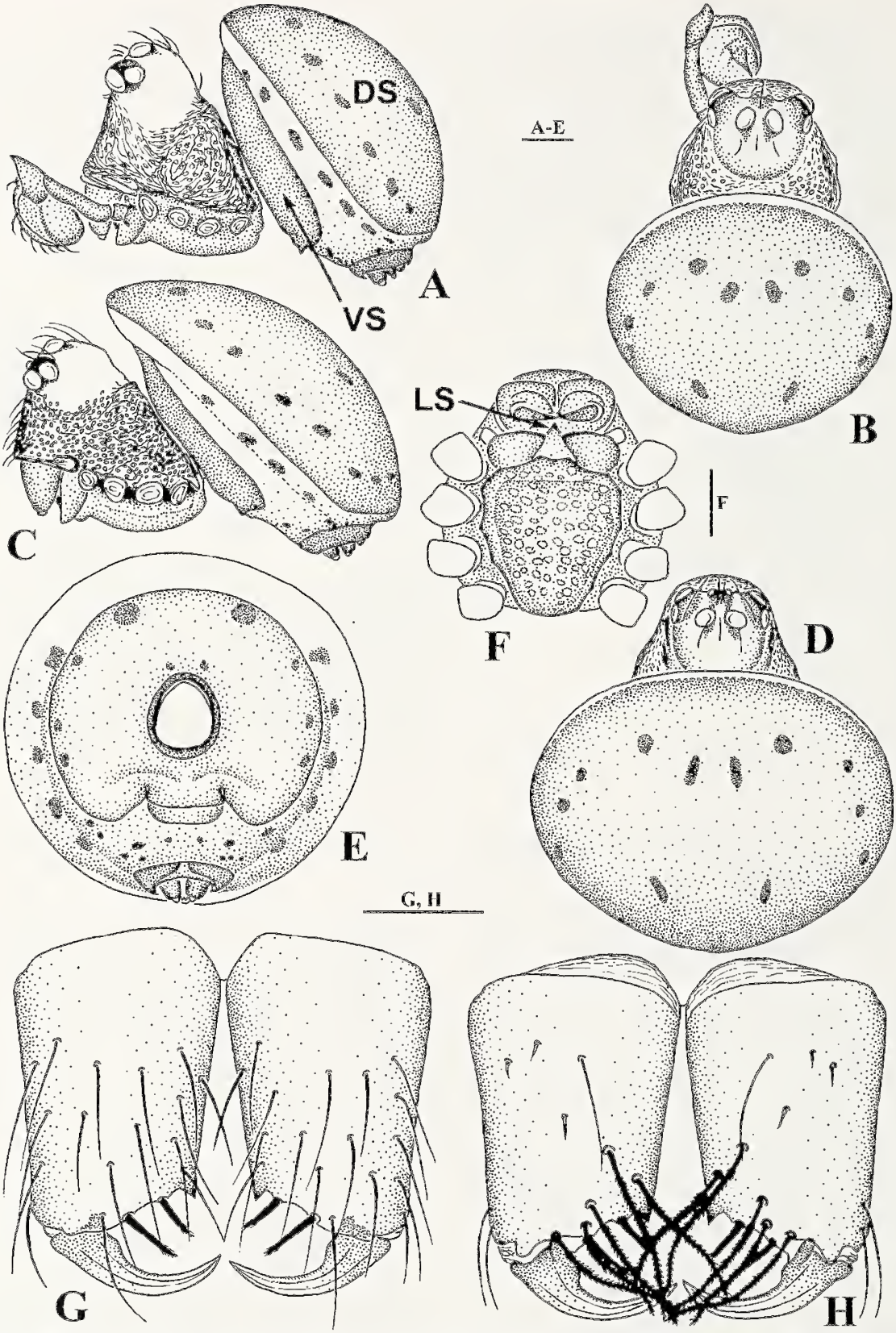

Figure 4.—*Minanapis menglunensis* **new species**, holotype male and paratype female from Yunnan. A. Male body, lateral; B. Same, dorsal; C. Female body, lateral; D. Same, dorsal; E. Female abdomen, ventral; F. Male sternum and coxa, ventral; G. Male chelicerae, anterior; H. Same, posterior. Scales: A–F = 0.10; G, H = 0.05.

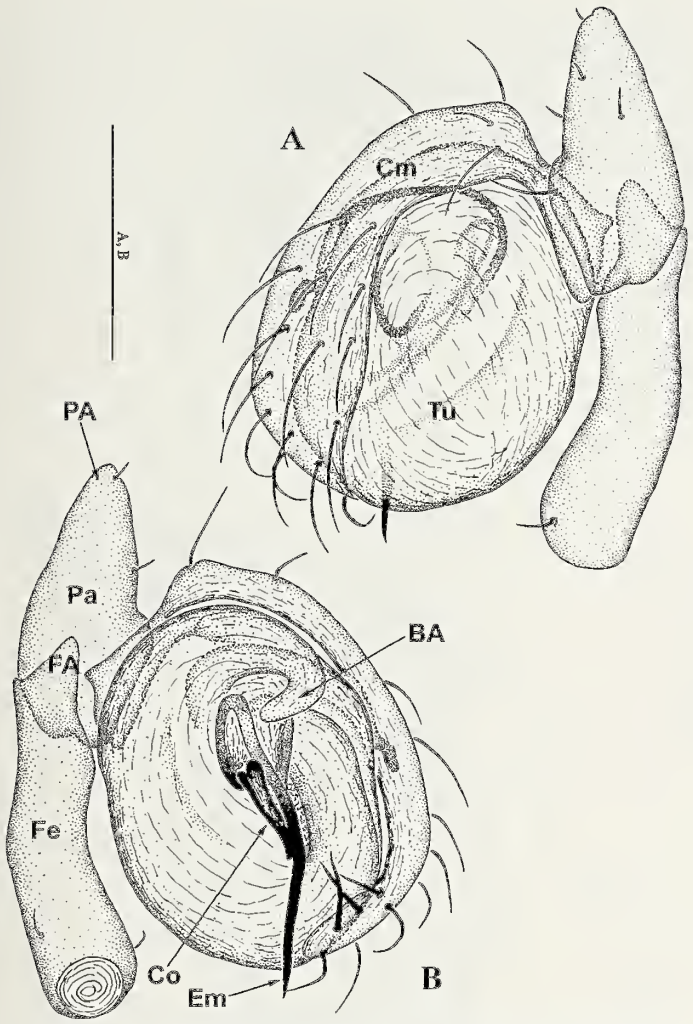

Figure 5.—*Minanapis menglunensis* **new species**, holotype male from Yunnan. A. Left palp, retrolateral; B. Same, prolateral. Scale = 0.10.

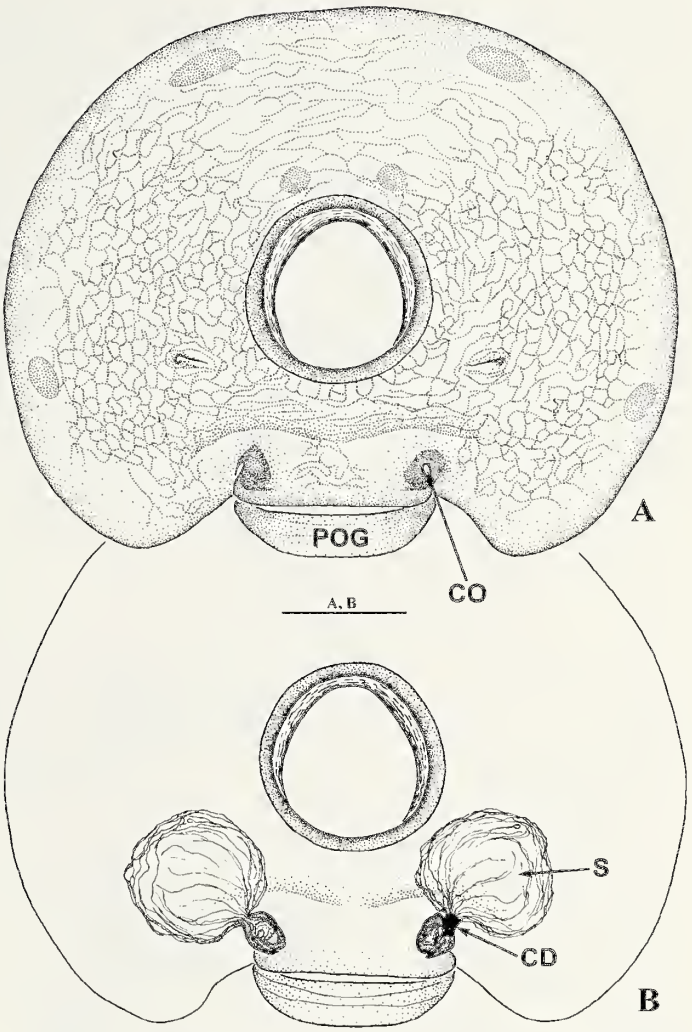

Figure 6.—*Minanapis menglunensis* **new species**, paratype female from Yunnan. A. Epigynum, ventral; B. Same, dorsal. Scale = 0.10.

corners of the carapace (Figs. 4A, C), female palpal segments extending beyond the coxae, presence of anterior book lungs (Figs. 4E, 6A), male palpal patella fused to tibia, and embolus extending far out from the palpal bulb (Figs 5A, B). It can be diagnosed from other *Minanapis* species by uncinate basal apophysis on palpal bulb, larger bulb, shorter conductor, needle-like embolus in the male (Figs. 5A, B), and by saccular, rather than sclerotized, spermathecae and very short copulatory ducts in the female (Figs. 6A, B).

Description.—*Male (holotype):* Total length 0.69. Carapace 0.34 long, 0.32 wide, 0.30 high. Clypeus 0.17 high. Sternum 0.19 long, 0.17 wide. Abdomen 0.48 long, 0.54 wide. Carapace brown, anterolateral depressions present and pore-bearing; cephalic area smooth, sharply elevated, apex at ocular area; thoracic area with modified pits and rugae on posterior margin. Eyes eight, round, white, in two rows; anterior median eyes smallest, anterior lateral eyes largest, posterior eyes subequal in size. Lateral eyes adjacent. In dorsal view, anterior and posterior eye row straight, equal width. Chelicerae short, slender, covered with long hairs anteriorly and five long plumose setae posteriorly, fang furrow with a promarginal tooth and two short setae. Labral spur small, triangular, not furcated. Sternum brown, modified with pits and reticulate, margin fused to carapace between all legs. Legs yellow-brown, patella with one dorsal spine distally, tibiae I and II with two spines and three trichobothria, tibiae III and IV with one spine and three trichobothria; each metatarsus with one trichobothrium. Leg measurements: I 0.87 (0.26, 0.11, 0.19, 0.11, 0.20); II 0.80 (0.24, 0.11, 0.16, 0.10, 0.19); III 0.71 (0.21, 0.10, 0.14, 0.09, 0.17); IV 0.76 (0.23, 0.10, 0.15, 0.10, 0.18). Leg formula: I-II-IV-III. Abdomen covered with paired sclerotized spots. Dorsal scutum round. Ventral scutum present. Spinneret area with an annular scutum. Palp simple. Palpal patella with a large distal apophysis, the patella fused to tibia. Bulb elliptic, median and apical apophysis absent, only with an uncinate basal apophysis. Embolus long, tube-shaped, situated medially on bulb, extending across and beyond distal tegulum. Conductor about one third of embolus in length. Cymbium with three spines prolateral-distally.

Female: Total length 0.75 (one of the paratypes). Carapace 0.37 long, 0.32 wide, 0.25 high. Clypeus 0.14 high. Sternum 0.20 long, 0.20 wide. Abdomen 0.57 long, 0.68 wide. Coloration and modification same as in male. Cephalic area slightly lower than in male. Pedipalp absent. Leg measurements: I 0.83 (0.25, 0.11, 0.18, 0.10, 0.19); II 0.77 (0.22, 0.11, 0.16, 0.10,

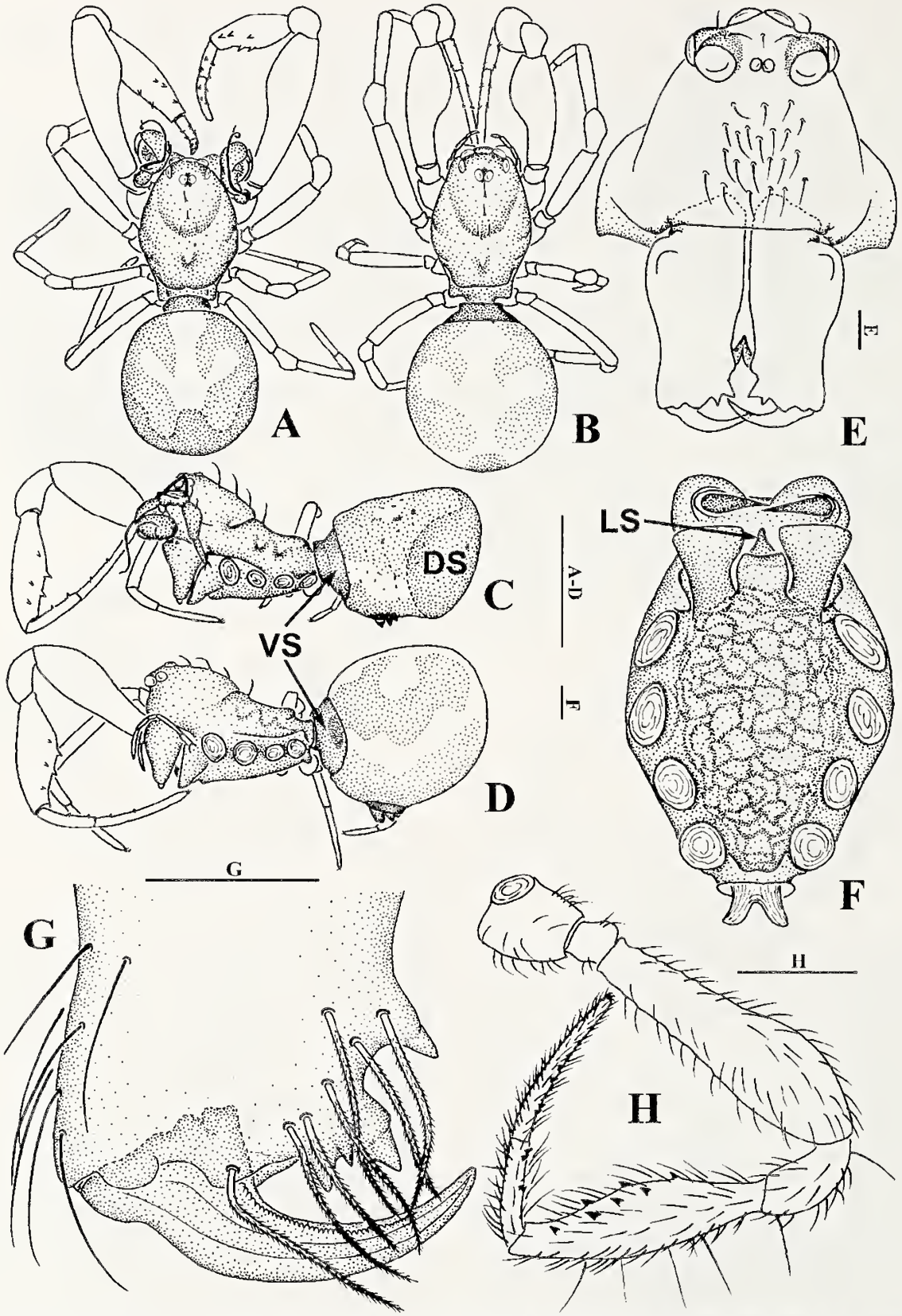

Figure 7.—*Sinanapis longituba* **new species**, holotype male and paratype female from Hainan. A. Male body, dorsal; B. Female body, dorsal; C. Same A, lateral; D. Same B, lateral; E. Male carapace and chelicerae, anterior; F. Male sternum, ventral; G. Left chelicera, posterior; H. Male left leg I, prolateral. Scales: A–D = 1.00; E, F = 0.10; H = 0.50.

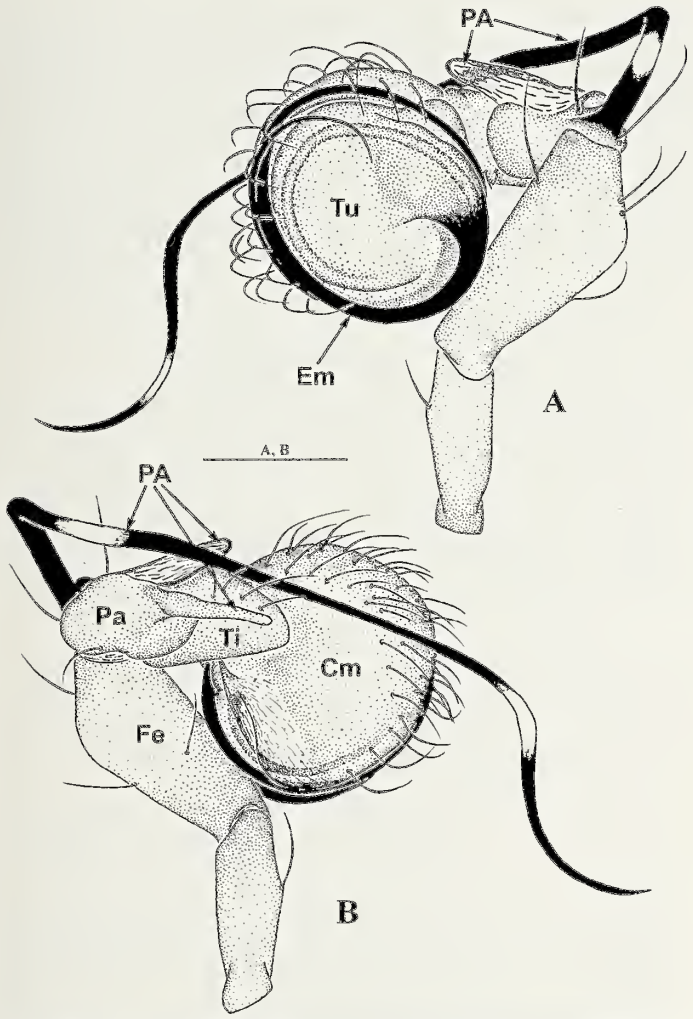

Figure 8.—*Sinanapis longituba* **new species**, holotype male from Hainan. A. Left palp, retrolateral; B. Same, prolateral. Scale = 0.20.

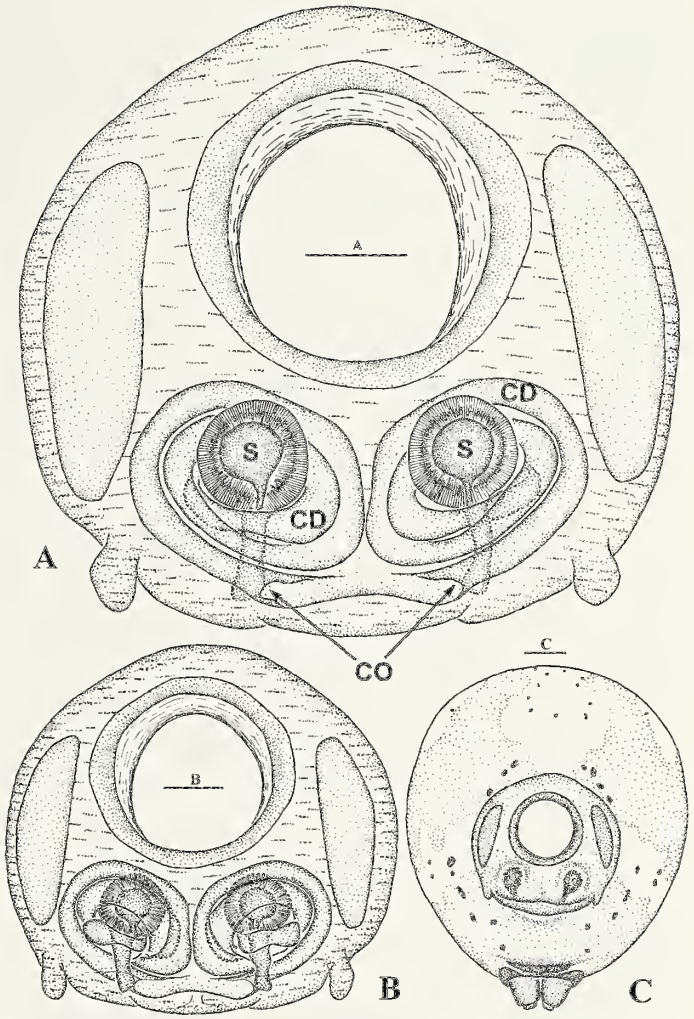

Figure 9.—*Sinanapis longituba* **new species**, paratype female from Hainan. A. Epigynum (treated in lactic acid), ventral; B. Same, dorsal; C. Abdomen, ventral. Scales: A, B = 0.10; C = 0.20.

0.18); III 0.67 (0.19, 0.10, 0.13, 0.09, 0.16); IV 0.76 (0.23, 0.10, 0.16, 0.10, 0.17). Leg formula: I-II-IV-III. Abdominal dorsal scutum round. Ventral scutum reticulate, with a pair of posterolateral corners. Epigynal area wrinkled, copulatory openings distinct. Spermathecae relatively large, saccular rugosed, connected to short, sclerotized copulatory duct; copulatory openings situated at posterior surface of epigynal shield.

Other material examined.—1 ♂, 3 ♀ (IZCAS), CHINA: *Yunnan*: Mengla County, Menglun Nature Reserve, primary tropical seasonal rainforest (21°55′N, 101°16′E), elevation 558 m, 22 July 2007, G Zheng. 5 ♂, 2 ♀ (IZCAS), Menglun Town, rubber-tea plantation (21°56′N, 101°17′E), elevation 561 m, 8–12 August 2006, G. Zheng.

Distribution.—Known only from the type locality (Fig. 10).

Genus *Sinanapis* Wunderlich & Song 1995

Sinanapis longituba **new species**

Figs. 7–10

Type material.—Holotype ♂ (IZCAS), CHINA: *Hainan*: male, Qiongzhong County, Mt. Limushan Nature Reserve (19°11′N, 109°44′E), elevation 655 m, 12 August 2007, S. Li and C. Wang. Paratypes: 3 ♂, 11 ♀ (IZCAS), same data as for holotype.

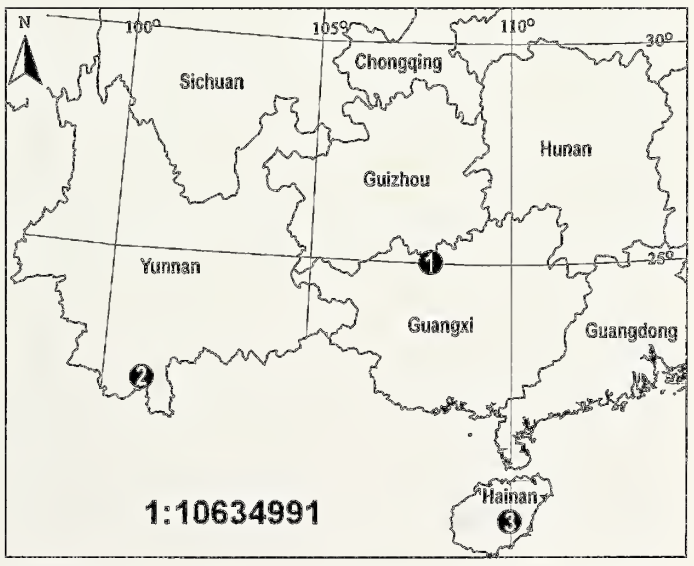

Figure 10.—Locality records of three new Chinese anapids. 1. *Gaiziapis encunensis* **new species**; 2. *Minanapis menglunensis* **new species**; 3. *Sinanapis longituba* **new species**.

Etymology.—Specific epithet is derived from Latin "*longitubus*"—long-tube, referring to the presence of a long process on palpal patella of male; adjective.

Diagnosis.—The new species is similar to *Sinanapis crassitarsus* Wunderlich & Song 1995 and *S. thaleri* Ono 2009 in having similar body size, strong male leg I, modified cusps on metatarsus and tarsus I, and complicated patellar apophyses in the male (Figs. 7A–D, H; 8A, B), but can be distinguished from *S. crassitarsus* and *S. thaleri* by the presence of anterior median eyes (Fig. 7E), very long and sclerotized, rather than grater-like, patellar apophysis on the palp, conical bulb with coiled embolus in the male (Figs. 8A, B). Female (unknown in *S. crassitarsus* and *S. thaleri*) can be easily recognized by the longer than wide book lung covers and the two coiled copulatory ducts encircled by spherical spermathecae (Figs. 9A–C).

Description.—*Male (holotype):* Total length 2.52. Carapace 1.20 long, 0.80 wide, 0.68 high. Clypeus 0.40 high. Sternum 0.90 long, 0.45 wide. Abdomen 1.20 long, 1.14 wide. Carapace red-brown, anterolateral depressions absent. Thoracic region modified with semiround pits, rugose at posterior margin; cephalic pars distinctly raised, smooth, with four setae on midline. Eight eyes in four diads, anterior median eyes smallest, anterior laterals largest, anterior median eyes separated by their diameter, posterior median eyes contiguous, larger slightly than posterior laterals in diameter. Lateral eyes adjacent. From dorsal view, anterior eye row straight, posterior eye row procurved. Chelicerae brown, with a proximally lateral knob, fang furrow with three isolated large promarginal teeth, one small retromarginal tooth and seven plumose setae posteriorly. Labral spur large, furcated at base. Sternum brown, modified with pits and reticulate. Legs brown-yellow, strong, femur I and tibia I swollen, tibia, metatarsus and tarsus of leg I with paired spurs ventrally; each patella distally with one dorsal spine and as well as on proximal of each tibia. Each tibia with four trichobothria. Leg measurements: I 4.26 (1.35, 0.48, 1.12, 0.53, 0.78); II 3.03 (0.90, 0.37, 0.71, 0.40, 0.65); III 2.15 (0.65, 0.25, 0.48, 0.30, 0.47); IV 2.57 (0.80, 0.27, 0.65, 0.36, 0.49). Leg formula: I-II-IV-III. Abdomen darkish, with a large trifoliate-light speckle on dorsum, dorsal scutum on rear, sclerotized spots on laterals. Ventral scutum present. Spinnerets with an annular sclerotized plate. Palp relatively large. Palpal trochanter subequal to two-thirds of palpal femur in length. Palpal femur swollen at one-third distally. Palpal patella with three apophyses, the retrolateral one very long and the prolateral two short. Palpal tibia without any apophysis. Palpal bulb simple, cone-shaped, without any apophyses. Embolus long, strongly sclerotized, coiled into two loops. Tegulum smooth and flat. Cymbium nearly funnelled, with long setae on the brim.

Female: Total length 2.43 (one of the paratypes). Carapace 1.12 long, 0.70 wide, 0.53 high. Clypeus 0.22 high. Sternum 0.87 long, 0.50 wide. Abdomen 1.30 long, 1.08 wide. Coloration and modification of body are same as in male, but abdominal dorsal scutum absent. Palp present and segmented. Leg chaetotaxy and eye pattern same as in male. Cephalic pars slightly lower than in male. Leg measurements: I 3.69 (1.15, 0.44, 0.95, 0.45, 0.70); II 2.70 (0.80, 0.33, 0.62, 0.35, 0.60); III 1.99 (0.55, 0.25, 0.43, 0.28, 0.48); IV 2.56 (0.78, 0.27, 0.61, 0.35, 0.55). Leg formula: I-II-IV-III. Abdominal patterns as in male. Ventral scutum modified by tiny stripes, with a pair of posterolateral corners. Book lung covers large, longer than wide. Spinneret area with an annular sclerotized plate. Spermathecae round, strongly sclerotized. Copulatory ducts long, coiled into two rings, opening at the posterior margin of epigynal area. Fertilization ducts short and straight, arising from the bottom of spermathecae.

Distribution.—Known only from the type locality (Fig. 10).

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Extreme mitochondrial population subdivision in southern Appalachian paleoendemic spiders (Araneae: Hypochilidae: *Hypochilus*), with implications for species delimitation

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Abstract. A prior study of molecular phylogenetic relationships in southern Appalachian *Hypochilus* taxa revealed unusually high intraspecific mitochondrial sequence divergences, but was limited by small intraspecific sample sizes. A subsequent in-depth population genetic study focused on a single species (*H. thorelli* Marx 1888), revealing genetic patterns consistent with extremely limited female-based gene flow among rock-outcrop limited populations. Here we extend the study of mitochondrial population genetic structuring to four remaining Appalachian *Hypochilus* species. Genetic inferences are based on a sample of COI mitochondrial sequences generated for over 250 specimens from 85 sampled locations. This geographic sample comprehensively covers the geographic distributions of all described taxa. Phylogenetic, network-based, and genealogical sorting index analyses reveal ubiquitous genetic structuring in all *Hypochilus* taxa. A majority of sampled locations possess limited genetic variation, with site-specific haplotypes forming genealogically exclusive "microclades", consistent with limited female-based gene flow at the spatial scales sampled. At deeper phylogenetic levels, four of five described species are recovered as monophyletic on mitochondrial gene trees. *Hypochilus pococki* Platnick 1987 is recovered as paraphyletic, and is fragmented into five genetically divergent, allopatric phylogroups. These phylogroups, and multiple clades within one of the *H. pococki* phylogroups, are also recovered as distinct clusters in a generalized mixed Yule-coalescent (GMYC) analysis, suggesting the possibility of multiple cryptic species in the Appalachian fauna. However, a qualitative survey of male palpal variation fails to reveal morphological differences that distinguish these highly divergent genetic lineages. We suggest that a nuclear gene tree perspective is ultimately needed to resolve this contrast.

Keywords: Cryptic species, genealogical sorting index, GMYC model, population subdivision

The uplands that comprise the several physiographic provinces of the southern Appalachian Mountains are ancient. Uplifted during the Paleozoic, highlands of this erosional landscape have been available for biotic evolution throughout the Cenozoic. Some authors contend that certain elements of the modern fauna in fact have histories that reach to the Mesozoic or Paleozoic eras (Dillon & Robinson 2009). More recently, the region has been impacted by climatic variation, and it is hypothesized that the southern Appalachians served as refugia for many taxa during the Pleistocene glaciations (e.g., Church et al. 2003; Crespi et al. 2003; Walker et al. 2009). This combination of climatic variability and long-term availability, in concert with high topographic complexity, has fostered remarkable in situ evolutionary diversification. The southern Appalachians today represent one of the most biodiverse regions in the northern hemisphere (Stephenson et al. 1993; Stein et al. 2000), comprising a hotspot for short-range endemic aquatic and upland taxa. In upland animal taxa, endemic radiations are seen, for example, in millipedes (Marek & Bond 2006, 2009; Marek 2010), spiders (Hedin 1997; Hendrixson & Bond 2005), harvestmen (Thomas & Hedin 2008; Hedin & Thomas 2010), salamanders (Crespi et al. 2003; Weisrock et al. 2006; Kozak & Wiens 2010), and many other cryophilic groups.

The spider genus *Hypochilus* is one of the most distinctive spider groups in North America, representing the most early-diverging lineage (Family Hypochilidae) of "true" spiders (Platnick 1977; Forster et al. 1987; Platnick et al. 1991). *Hypochilus* shows a fragmented continental distribution, with species found in the southern Rocky Mountains, montane areas of California and the southern Appalachian Mountains (Catley 1994; Hedin 2001). The monophyletic southern Appalachian fauna (Catley 1994; Hedin 2001) includes five described species (*H. gertschi* Hoffman 1963, *H. thorelli* Marx 1888, *H. pococki* Platnick 1987, *H. sheari* Platnick 1987 and *H. coylei* Platnick 1987) distributed in strict allopatry across six states, from northern Alabama and Georgia to West Virginia (Fig. 1). Several lines of evidence suggest that Appalachian *Hypochilus* are both ecologically and morphologically conservative. All eastern species prefer relatively mesic habitats, and are almost always found on rock outcrops, where they build distinctive "lampshade" webs. Different species are sometimes found in adjacent locations on the same geologic outcrop (e.g., *H. thorelli* and *H. pococki* on Cumberland Mountain in southwest Virginia; Fig. 1), but multiple species have never been collected at the same site, indicating that ecological similarity (niche conservatism) may preclude syntopy. Appalachian *Hypochilus* are extremely similar in somatic morphology, distinguished only by subtle differences in male and female genital morphology (Forster et al. 1987; Huff & Coyle 1992; Catley 1994).

Prior research clearly shows that these spiders are also dispersal limited. Based on sparse phylogeographic sampling, Hedin (2001) revealed deep mitochondrial divergences within Appalachian species. Hedin and Wood (2002) conducted a more thorough mitochondrial study of *H. thorelli*, revealing high intraspecific mitochondrial divergences and fractal genetic structuring. Mitochondrial sequences from all sampled locations formed genealogically exclusive clades, regardless of the geographic proximity of sample sites. Although no quantitative morphological assessment was conducted, the authors noted no differences in genitalic morphology between

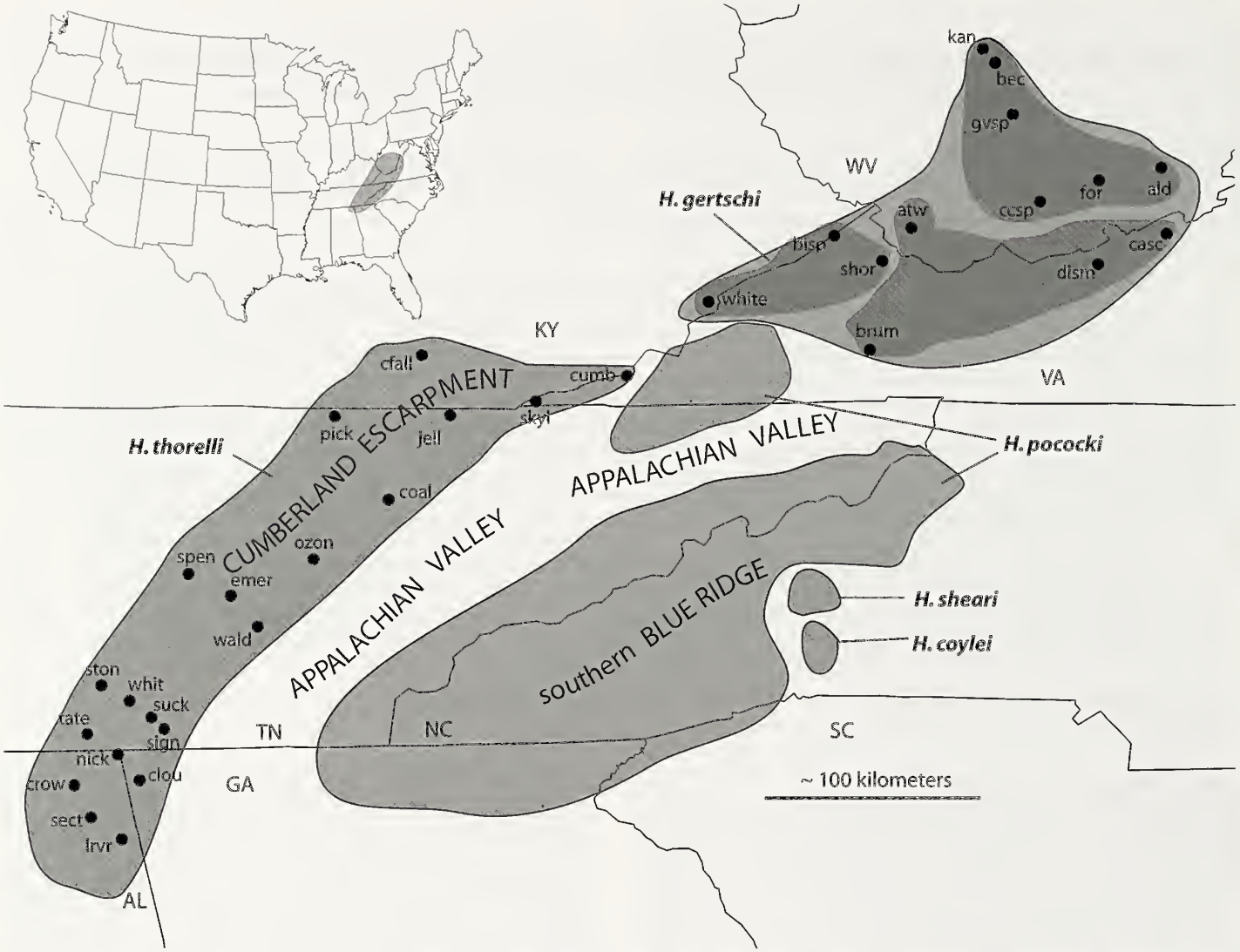

Figure 1.—Map of southern Appalachian region showing physiographic provinces and general distribution of eastern *Hypochilus*, with sampled sites for *H. thorelli* and *H. gertschi* (site acronyms found in Table 1). Geographic subclades consistently recovered in alternative RAxML analyses indicated by darker shading for *H. gertschi*.

populations, providing further evidence for morphological conservatism (i.e., morphological cohesion despite limited female-based gene flow).

Here we extend our studies of mitochondrial population structure and phylogeography to all described species of Appalachian *Hypochilus*, addressing two primary questions regarding genetic population structure and divergence. First, using a large genetic sample we investigate whether other Appalachian *Hypochilus* species show nearly complete mitochondrial population subdivision, as observed in *H. thorelli*. Appalachian taxa share many biological similarities, but also differ in important ways that might impact patterns of genetic structuring (e.g., relative range size, latitudinal position, etc., Fig. 1). Second, we use mitochondrial sequence data to detect possible cryptic species lineages within the Appalachian *Hypochilus* fauna. To address this second question we use standard gene tree patterns (e.g., do nominate taxa form genetic clades?), combined with methods of species delimitation derived from coalescent theory. For "candidate" cryptic lineages we also qualitatively assess geographic variation in male palpal morphology.

METHODS

Sampling.—Specimens representing the five Appalachian species were collected as follows: *H. pococki* (159 individuals from 56 sites), *H. gertschi* (61 individuals/13 sites), *H. sheari* (21 individuals/8 sites), *H. coylei* (18 individuals/6 sites) and *H. thorelli* (2 individuals/2 sites) (Figs. 1, 2; Table 1). DNA sequences gathered from these specimens were combined with previously collected data (Hedin 2001; Hedin & Wood 2002): *H. pococki* (4 individuals/4 sites), *H. gertschi* (2 individuals/2 sites), *H. sheari* (2 individuals/2 sites) and *H. thorelli* (18 individuals/18 sites). Collecting locations were approximately uniformly spread over the known range of each species, with a majority of neighboring sites separated by 20–40 km. Species with smaller distributions were sampled at a finer geographic scale (e.g., *H. coylei* sites separated by ~10 km). At any given site, specimen collection was dispersed (e.g., different regions

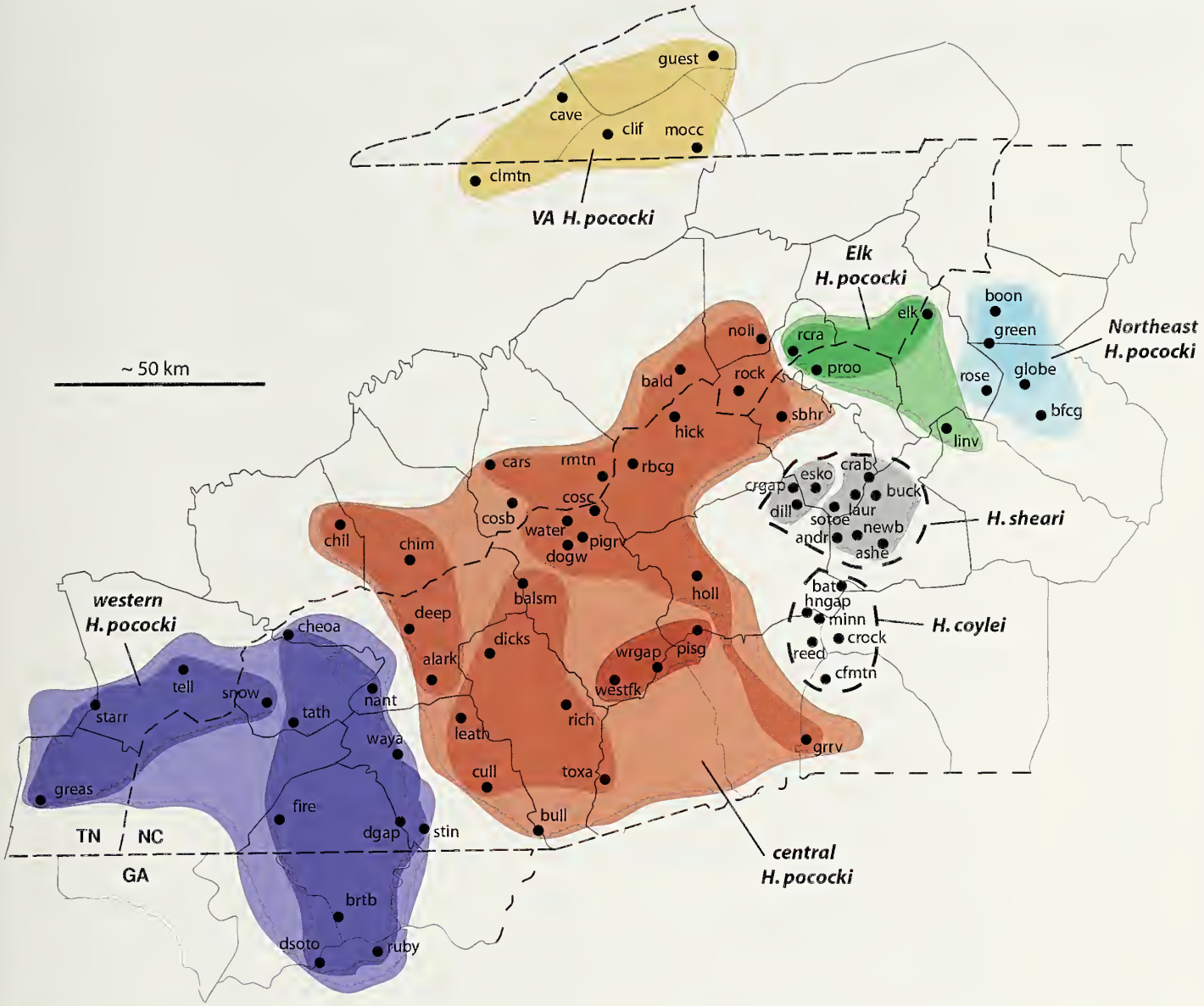

Figure 2.—Map of sampled sites for *H. pococki, H. sheari,* and *H. coylei,* mostly in the southern Blue Ridge Province. Site acronyms are found in Table 1. Primary geographic clades of *H. pococki,* as recovered in phylogenetic analyses, are individually colored. Geographic subclades consistently recovered in alternative RAxML analyses indicated by darker shading.

of a rock face) as we attempted to reduce the probability of collecting related individuals. Specimens intended for molecular work were preserved in 100% EtOH in the field. Because there are no known instances of species sympatry in eastern *Hypochilus* (Catley 1994), we sometimes used immature specimens for genetic analysis; immature specimens were always associated with a sample of adult voucher specimens (preserved in 80% EtOH) from the same location. Adult specimens were identified to species using diagnostic characters following Forster et al. (1987), Huff and Coyle (1992) and Catley (1994). Voucher specimens for all species, and all major phylogeographic clades within species (see Results), have been deposited at the California Academy of Sciences.

Molecular techniques.—Genomic DNA was extracted from leg tissues using the DNeasy Kit (Qiagen). Genomics were used as templates in PCR (Polymerase Chain Reaction) experiments, targeting an approximately 900 bp fragment of the mitochondrial cytochrome oxidase I (COI) gene region. This is the same gene region used by Hedin and Wood (2002), allowing a direct comparison between datasets. This gene region also overlaps the "DNA barcoding" locus used for spiders by Robinson et al. (2009). PCR experiments included an initial 94°C denaturation followed by 30 cycles of 45 s at 94°C, 45 s at 45°C, 90 s at 72°C, with a final 10-min extension at 72°C. Primers utilized are shown in Table 2. All PCR experiments included *Ex Taq* (Takara Bio, Inc.) with manufacturer-provided dNTP mixture and *Ex Taq* buffer (Mg^{2+}). PCR amplification products were purified via Polyethylene Glycol (PEG) precipitation, or by using an *IsoPure* PCR Purification and Gel Extraction Kit (Denville Scientific, Inc.). PCR products were sequenced using Big Dye Version 3 dye chemistry (ABI) on ABI 377 and Prism 3100

Table 1.—Taxon identity, locality information, site acronym, genetic diversity (observed maximum number of nucleotide site differences per geographic location), specimen number(s), and GenBank accession numbers. Bolded voucher number sequences submitted to GenBank. Adult male spiders were examined from those sites with site acronyms highlighted by an asterisk.

Species	Locality	Acronym	Max diff.	Hedin Lab #	GenBank acces. no.
H. pococki "central"	TN: Cocke Co., SE Round Mountain, W Rattlesnake Gap, 35.8471, −82.9443	rmtn	1	**H350=H351**, H352	JQ974835
"	NC: Madison Co., Hwy 209, W Rocky Bluff CG @ Long Mtn Branch, 35.8599, −82.8502	rbcg	7	H356, H359, **H360**	JQ974836
"	NC: Madison Co., East Prong Hickory Fork Creek, 35.9900, −82.52448	hick	12	H372, **H373=H375**	JQ974837
"	TN: Greene Co., Bald Mtns, E Greystone Mtn, Round Knob Road, 36.0799, −82.6859	bald	2	**H678=H679**, H680	JQ974838
"	NC: Polk Co., Green River Cove Road, 35.2539, −82.3301	grvr	0	**H489=H491=H492**	JQ974839
"	TN: Unicoi Co., E Rocky Fork, just up Edwards Branch road, 36.0662, −82.5245	rock	7	H377, H378, **H379**	JQ974840
"	TN: Washington Co., Little German Rd, along Nolichucky River, 36.1680, −82.4675	noli	1	**H694=H695**, H696	JQ974841
"	NC: Yancey Co., 19W, along Cane River, vic. Snakebite Holler Road., 35.9496, −82.3837	*sbhr	1	H674, **H673=H675**	JQ974842
"	NC: Haywood Co., Cold Springs Creek, 35.7594, −82.9953	cosc	1	H344, **H345=H346**	JQ974843
"	NC: Haywood Co., Dogwood Flats Creek, W Longarm Mtn, 35.7201, −83.0731	*dogw	4	H333, **H332=H334**	JQ974844
"	NC: Haywood Co., S. Waterville, Flat Branch Crk (of Mt Sterling Crk), 35.7407, −83.0741	water	6	H328, **H326=H327=H329**	JQ974845
"	NC: Haywood Co., FR 288 above Pigeon River, 35.7260, −83.0265	pigrv	2	**H338=H339**, H340	JQ974846
"	TN: Cocke Co., Carson Springs Road, 35.9411, −83.2567	cars	1	H689, **H690=H691**	JQ974847
"	NC: Jackson Co., Dicks Creek, near Dicks Creek Church, 35.4056, −83.2586	dicks	1	**H597=H598**, H599	JQ974848
"	NC: Swain Co., GSMNP, road to Balsam Mtn, N Black Camp Gap, 35.5437, −83.1679	balsm	7	H629, H630, **H631**	JQ974849
"	NC: Transylvania Co., near Toxaway Falls, 35.1247, −82.9297	*toxa	0	**H655=H656=H657**	JQ974850
"	NC: Jackson Co., Rich Mtn, SE Sugar Creek Gap, 35.2907, −83.0040	rich	3	H608, H609, **H610**	JQ974851
"	NC: Macon Co., NE Leatherman, 35.2965, −83.3666	leath	1	H664, **H666**	JQ974852
"	TN: Sevier Co., GSMNP, Chimneys @ Hwy 441, 35.6417, −83.4818	chim	–	–	AF303511
"	NC: Swain Co., GSMNP, Deep Creek, 35.4644, −83.4344	deep	0	**H623=H624=H625**	JQ974853
"	TN: Blount Co., Chilhowee Mountain, near Walland, 35.7331, −83.8165	*chil	1	H706, **H705=H707**	JQ974854
"	NC: Swain Co., Alarka Road, N Deep Gap church, 35.3482, −83.4064	alark	1	H594, **H593=H595**	JQ974855
"	NC: Transylvania Co., Hwy 276, 2 mi. S BRP, S Wagon Road Gap, 35.3682, −82.7862	wrgap	5	H500, **H501**	JQ974856
"	NC: Buncombe Co., NE Mt. Pisgah, head of McKinney Creek, 35.4448, −82.7225	pisg	0	**H639=H640**	JQ974857
"	NC: Haywood Co., Hwy 215, near head West Fork Pigeon river, 35.3390, −82.9016	*westfk	1	H635, **H633=H636**	JQ974858
"	NC: Macon Co., Chattooga River @ Bullpen bridge, 35.0172, −83.1262	bull	1	H613, **H615=AF303512**	AF303512
"	TN: Cocke Co., GSMNP, trail from Cosby to Low Gap, 35.7374, −83.1813	cosb	18	H322, **H321=H323**	JQ974859
"	NC: Macon Co., Cullasaja River Gorge, 35.0803, −83.2578	cull	2	H603, H604, **H605**	JQ974860
"	NC: Buncombe Co., Holland Mtn., Dogwood Road, S of Newfound, 35.6008, −82.7259	holl	5	H770, H773	JQ974861

Table 1.—Continued.

Species	Locality	Acronym	Max diff.	Hedin Lab #	GenBank acces. no.
H. pococki "Virginia"	VA: Lee Co., Cave Spring Recreation Area, NE Penington Gap, 36.8033, −82.9210	cave	0	**H232=H233**	JQ974862
"	VA: Wise Co., above Guest River, 36.9009, −82.4147	*guest	1	H742, **H740=H743**	JQ974863
"	VA: Scott Co., Cliff Mtn, 36.7495, −82.7787	*clif	0	**H733=H734=H735**	JQ974864
"	TN: Hancock Co., Hwy 31 on Clinch Mtn, 36.413, −83.2237	*clmtn	7	**H754**, H756	JQ974865
"	VA: Scott Co., Hwy 23/58/421 @ Moccasin Gap, 36.6338, −82.5550	*mocc	–	**H763**	JQ974866
H. pococki "Northeast"	NC: Watauga Co., West of Boone @ Watauga Rvr Crossing, Hwy 194, 36.1943, −81.7451	boon	1	H411, **H410=H413**	JQ974867
"	NC: Watauga/Caldwell Co., Green Mtn., Hwy 221 @ Green Mtn. Creek, 36.1142, −81.7782	*green	0	**H426=H427=H428**	JQ974868
"	NC: Avery Co., Roseboro Road, past first crossing Rockhouse Crk, 36.0192, −81.7813	*rose	0	**H432=H433=H434**	JQ974869
"	NC: Caldwell Co., Boone Fork CG, S of Chestnut Mtn, 36.0071, −81.6166	*bfcg	1	**H416=H417, H418**	JQ974870
"	NC: Caldwell Co., Globe Mountain Road, near Globe Mtn gap, 36.029, −81.667	*globe	0	**H422=H424=H425**	JQ974871
H. pococki "western"	NC: Graham Co., along Snowbird Creek, near Wilson Cabin, 35.2733, −83.9051	snow	0	**H572=H573=H574**	JQ974872
"	NC: Graham Co., Snowbird Mtns, N Tatham Gap, head Long Creek, 35.2579, −83.8196	tath	1	**H577=H578, H579**	JQ974873
"	NC: Swain Co., GRSMNP, along Lake Cheoah, Hwy 28, 35.4644, −83.8866	*cheoa	0	**H582=H584=H585**	JQ974874
"	TN: Polk Co., Hwy 64, along Lake Ocoee, 0.25 mi. E Greasy Crk bridge, 35.1112, −84.5647	*greas	0	**H546=H547=H548**	JQ974875
"	NC: Macon Co., W Wayah Depot, 35.1594, −83.5271	*waya	14	H643, **H644=H645**	JQ974876
"	NC: Clay Co., Fires Creek, W Omphus Ridge, 35.1029, −83.8435	fire	2	**H526=H527**, H528	JQ974877
"	GA: Towns Co., road to Brasstown Bald, 34.8593, −83.8008	brtb	19	H531, H533, **H534**	JQ974878
"	GA: White Co., Anna Ruby Falls Rec Area, 34.7576, −83.7101	*ruby	0	H536=H539=H540	JQ974879
"	GA: Lumpkin Co., DeSoto Falls Rec Area, trail to Upper Falls, 34.7062, −83.9153	dsoto	16	H541, H542, **H543**	JQ974880
"	TN: McMinn Co., N end of Starr Mountain, 35.3420, −84.4076	starr	3	H551, H553, **H554**	JQ974881
"	TN: Monroe Co., Tellico River, near Bald River Falls, 35.3248, −84.1787	tell	10	H556, **H558=H559**	JQ974882
"	NC: Swain Co., Nantahala River Gorge, 0.2 mi NE Blowing Spring, 35.32347, −83.63085	nant	1	H505=H506=H507	AF303513
"	NC: Macon Co., 4.3 mi S Standing Indian CG, 35.0347, −83.5057	*stin	0	**H512=H513=H514**	JQ974883
"	NC: Macon Co., 0.2 mi. N Deep Gap, 35.0425, −83.5550	dgap	0	**H517=H518=H519**	JQ974884
H. pococki "Elk"	NC: Burke Co., Linville Gorge, opposite Bull branch, 35.9396, −81.9219	*linv	6	H438, H437=H440	AF303514
	NC: Mitchell Co., Pigeonroost Creek, N of Nolichucky River, 36.0983, −82.2831	*proo	0	**H383=H387=H388**	JQ974885
"	NC: Avery Co., Elk River Cave, ~ 1 mi S Elk River Falls, 36.1892, −81.9617	*elk	2	H401, H402, **H403**	JQ974886
"	TN: Unicoi Co., Rock Creek Rec Area, 36.1379, −82.3482	*rcra	3	**H711**, H713	JQ974887
H. sheari	NC: Buncombe Co., W Cane River Gap, Hwy 197, 35.8036, −82.3536	crgap	0	**H444=H447=H448**	JQ974888
"	NC: Buncombe Co., Walker branch of Dillingham Creek 35.7677, −82.3594	*dill	3	H449, H450, **H451**	JQ974889

Table 1.—Continued.

Species	Locality	Acronym	Max diff.	Hedin Lab #	GenBank acces. no.
"	NC: McDowell Co., Newberry Crk (above Horse Br), N of Old Fort, 35.6825, −82.2170	newb	0	**H362=H363**	JQ974890
"	NC: Yancey Co., S. Big Laurel Mtn., N off BRP, 35.7401, −82.1991	*laur	0	**H364=H366**	JQ974891
"	NC: Yancey Co., South Toe River, below Chestnut knob, 35.7265, −82.2452	sotoe	6	**H370**, H371	JQ974892
"	NC: McDowell Co., Hwy 80, along Buck Creek, 35.7606, −82.1572	buck	0	**H454=H456=H457**	JQ974893
"	NC: McDowell Co., Andrew's Geyser, S side of Mill Creek, 35.6507, −82.2433	*andr	0	**H460=H461=H462**	JQ974894
"	NC: Yancey Co., Cane River, N Eskota, 35.8014, −82.3124	*esko	0	**H669=H670=H671**	JQ974895
"	NC: Yancey Co., Crabtree Falls	*crab	–	–	AF303515
"	NC: McDowell Co., US 70, E of Asheville	ashe	–	–	AF303516
H. coylei	NC: Buncombe Co., NW Hickory Nut Gap, Hwy 74, 35.4898, −82.3627	*hngap	2	H469, **H467=H468=H470**	JQ974896
"	NC: Rutherford Co., Chimney Rock Park, 35.4307, −82.2482	crock	1	**H473=H475**, H476	JQ974897
"	NC: Henderson Co., below Minnihaha Falls, Hwy 9, 35.4603, −82.2880	*minn	3	**H478=H481**, H479, H480	JQ974898
"	NC: Henderson Co., Reedypatch Creek, Hwy 64, W Little Fork Mtn, 35.4355, −82.3024	*reed	1	H484, H487=H488	JQ974899
"	NC: Polk Co., Cliffield Mountain, 35.3468, −82.2705	*cfmtn	1	H652, **H651=H653**	JQ974900
"	NC: Buncombe Co., below Round Mtn, Bat Cave road, 35.5314, −82.2202	*bat	–	H648	JQ975901
H. gertschi	VA: Washington Co., Brumley Creek @ Brumley Gap, 36.7933, −82.0229	brum	2	H728, **H729=H730**	JQ974902
"	VA: Buchanan Co., ~ 2 mi. W entrance Breaks Interstate SP, Hwy 80, 37.3012, −82.2880	bisp	6	H190, H191, H192, H193, **H194**	JQ974903
"	KY: Letcher Co., S Whitesburg, Hwy 199 @ summit of Pine Mtn, 37.0750, −82.8100	*white	8	**H236=H237**, H238=H239, H240	JQ974904
"	VA: Giles Co., Cascades of Little Stony Creek, ~ 2.5 mi. E of trailhead, 37.3643, −80.5792	casc	1	H221=H222, **H220=H223=H224 (=AF303519)**	AF303519
"	VA: Giles Co., Dismal Falls, 37.1878, −80.9003	dism	0	**H210=H211=H212=H213=H214=H215**	JQ974905
"	VA: Buchanan Co., 6 mi. W Shortt Gap, Hwy 460, along Levisa Fork, 37.1887, −81.9523	*shor	1	**H200=H201=H203, H204**	JQ974906
"	WV: McDowell Co., Hwy 83 @ Atwell, 37.3468, −81.7635	*atw	0	**H180=H181=H182=H183=H184**	JQ974907
"	WV: Fayette Co., Hwy 60, 0.5 mi. SW Kanawha Falls 38.1430, −81.2125	*kan	21	**H170=H171=H173**, H172=H174	JQ974908
"	WV: Fayette Co., ~ 1 mi. N Beckwith, along Laurel Creek, 38.1062, −81.1493	bec	0	**H160=H161=H162=H163=H164**	JQ974909
"	WV: Raleigh Co., W Grandview SP, 1 mi. E jnt Hwys 41/61, 37.8465, −81.1223	gvsp	1	**H150=H151=H152**, H153	JQ974910
"	WV: Mercer Co., Camp Creek SP, vic. Campbell Falls trailhead, 37.5092, −81.1337	*ccsp	1	H140=H141=H142=H143	AF303518
"	WV: Summers Co., E Forest Hill, along Spruce Run, 37.5906, −80.7913	for	0	**H130=H131=H132=H133=H134**	JQ974911
"	WV: Greenbrier Co., Rt 63 along Greenbrier River, 2 mi. E Alderson, 37.7308, −80.5955	ald	0	**H120=H121=H122=H123=H124**	JQ974912
H. thorelli	TN: Marion Co., Tate Spring Cave, se of Monteagle, 35.1770, −85.8073	tate	–	**H683**	JQ974913
"	VA: Lee Co., Cumberland Mtn, Wagonroad Tunnel Trail, 36.7308, −83.2207	cumb	–	**H802**	JQ974914
"	VA: Lee Co., Cumberland Gap NP, vic. Skylight Cave, 36.6165, −83.6443	*skyl	–	–	AF303510
"	KT: Whitley Co., Hwy 90, ~ 2 mi. E Cumberland Falls SP, 36.8474, −84.3083	cfall	–	–	AY102038

Table 1.—Continued.

Species	Locality	Acronym	Max diff.	Hedin Lab #	GenBank acces. no.
"	TN: Campbell Co., E of Jellico, Hwy 25W, 36.5756, −84.0691	*jell	–	–	AY102039
"	TN: Morgan Co., NW Coalfield, Hwy 62, Little Brushy Mtn, 36.0513, −84.4389	coal	–	–	AY102042
"	TN: Pickett Co., Pickett SF, Hwy 154 @ Natural bridge, 36.5452, −84.7976	pick	–	–	AY102043
"	TN: Cumberland Co., Ozone Falls, Hwy 70, 35.8805, −84.8103	*ozon	–	–	AF303509
"	TN: Van Buren Co., 0.5 mi. E Spencer, Hwy 30, 35.7319, −85.4321	spen	–	–	AY102046
"	TN: Bledsoe Co., Hwy 30 @ Emery Mill, W Pikeville, 35.6517, −85.1827	emer	–	–	AY102049
"	TN: Rhea Co., near Walden Ridge, Hwy 30 ~ 4 mi. W Dayton, 35.5298, −85.0495	wald	–	–	AY102050
"	TN: Grundy Co., Savage Gulf NA, Stone Door, 35.4397, −85.6487	*ston	–	–	AY102051
"	TN: Marion Co., ~ 5 mi. NW Whitwell, Hwy 108, ~ 1 mi S Star Gap, 35.2398, −85.5123	whit	–	–	AY102054
"	TN: Marion Co., Hwy 27, along Suck Creek, 35.1456, −85.3898	suck	–	–	AY102056
"	TN: Hamilton Co., Signal Mountain, vic. Chattanooga, 35.1193, −85.3477	sign	–	–	AF303508
"	GA: Dade Co., Cloudland Canyon SP, NW side Daniel Creek, 34.8343, −85.4843	clou	–	–	AY102061
"	AL: Jackson Co., Nickajack Cove, Hwy 73, 34.9804, −85.6094	nick	–	–	AY102063
"	AL: Jackson Co., Crow Mtn, below Clemmons Pt, Co. Rd 33, 34.8169, −86.0258	*crow	–	–	AY102064
"	AL: Jackson Co., NE side of Section, Hwy 35, 34.5955, −85.9981	sect	–	–	AY102066
"	AL: DeKalb Co., Little River Canyon, 34.3642, −85.6599	lrvr	–	–	AY102067
H. bonnetti	CO: Fly Cave				AF303525
H. kastoni	CA: West Boulder Lake				AF303521
H. bernardino	CA: Camp Creek				AF303524

capillary machines. Sequence contigs were assembled and edited using Sequencher version 4.2.2, and manually aligned using MacClade version 4.06 (Maddison & Maddison 2003). Sequence alignment was trivial, as no indels were present. Published COI sequences of *H. bonnetti* Gertsch 1964 (AF303525) from Colorado, as well as *H. kastoni* Platnick 1987 (AF303521) and *H. bernardino* Catley 1994 (AF303524) from California were used to root phylogenetic trees (sequences from Hedin 2001).

Phylogenetic and network analysis.—Identical haplotypes, except those shared among collection sites (less than five total haplotypes), were merged in MacClade prior to phylogenetic analysis. Gene trees were estimated using maximum likelihood (ML); rapid ML searches were conducted using RAxML version 7.0.4 (Stamatakis et al. 2008), implemented through the CIPRES (Cyberinfrastructure for Phylogenetic Research) portal v1.13. Searches included 100 rapid bootstrap replicates with a subsequent thorough ML search, assuming a GTR + G model. To explore alternative partitioning strategies, three separate RAxML analyses were conducted [unpartitioned, 2 partitions (first plus second, third), 3 partitions (first, second, third positions)]. For a subset of closely-related sequences that showed patterns of haplotype sharing among collection sites (see Results), haplotype networks were constructed using the program TCS v. 1.21 (Clement et al. 2000).

Genealogical sorting index.—The genealogical sorting index (*gsi*) statistic (Cummings et al. 2008) was used to quantify the degree of genealogical clustering of COI sequences for a priori labeled groups. Values of this statistic lie on a continuum, with values of 0 indicating a random geographic distribution of sequences, and values of 1 indicating complete exclusive ancestry. We used collecting localities as a priori grouping variables; exclusive ancestry of COI sequences collected from a focal location implies limited (or non-existent) female-based gene flow among sampled locations. All analyses were conducted using the *gsi* website (http://www.genealogicalsorting.org/), with statistical significance assessed using 10,000 permutations of group labels on a fixed tree topology. The ML tree resulting from a no partitions RAxML analysis of an "all haplotypes" matrix (i.e., duplicate haplotypes not collapsed) was used as an input tree.

Yule-coalescent species delimitation.—The generalized mixed Yule-coalescent (GMYC) model (Pons et al. 2006; Monaghan et al. 2009) was used to identify genealogical clusters that may also correspond to cryptic species lineages. This model relies upon an expected difference in branching time intervals

Table 2.—PCR primer information. Primer references as follows: C1-J-1751SPID, C1-N-2568, C1-N-2776 (Hedin & Maddison 2001); C1-J-1718 (Simon et al. 1994); C1-J-1598HYPO, C1-J-1751MG, C1-J-1751SHE, C1-J-1751CO, C1-N-2568TH (this study). Primers marked with an asterisk were used in sequencing reactions.

PCR Primers	Taxon
*C1-J-1598HYPO, 5′-CGRGTWGAGTTRGGGCAAGT-3′	*H. pococki, H. sheari, H. coylei*
*C1-J-1718, 5′-GGAGGATTTGGAAATTGATTAGTTCC-3′	*H. pococki, H. sheari, H. coylei*
*C1-J-1751SPID, 5′-GAGCTCCTGATATAGCTTTTCC-3′	*H. thorelli*
*C1-J-1751MG, 5′-GGAGCTCCCGATATGGCGTTCCC-3′	*H. pococki, H. sheari, H. coylei*
*C1-J-1751CO, 5′-GGAGCGCCGGATATAGCGTTTCC-3′	*H. pococki, H. sheari*
C1-J-1751SHE, 5′-GGAGCACCAGAYATAGCATTTCC-3′	*H. pococki, H. sheari*
*C1-N-2568, 5′-GCTACAACATAATAAGTATCATG-3′	*H. pococki, H. sheari, H. coylei*
*C1-N-2568TH, 5′-GCCACAACGTAATAAGTATC-3′	*H. pococki, H. sheari*
*C1-N-2776, 5′-GGATAATCAGAATATCGTCGAGG-3′	*H. pococki, H. sheari*

between species (modeled as a stochastic birth-only Yule process) as compared to branching time intervals within species (modeled as a neutral coalescent process). Maximum likelihood is used to fit the GMYC model to an ultrametric tree to identify a threshold time (T) that corresponds to the Yule-coalescent transition (i.e., speciation). The model has been extended to allow multiple threshold times in a single phylogeny (see Monaghan et al. 2009) and has been used in many species delimitation studies in arthropods (e.g., Pons et al. 2006; Papadopoulou et al. 2008; Monaghan et al. 2009; Vuataz et al. 2011; Hamilton et al. 2011).

The three-partitions RAxML tree was used as input in GMYC analyses conducted using statistical packages implemented in R version 2.13.0. The *chronopl* function was used to transform the RAxML tree to an ultrametric tree using penalized likelihood (Sanderson 2002), and the *multi2di* function was used to randomly resolve polytomies in the ultrametric tree. Both functions are implemented in the APE library, version 2.5.3 for R (Paradis et al. 2004; Paradis 2006). Single and multiple-threshold GMYC models were optimized using the R script available within the SPLITS package (http://r-forge.r-project.org/projects/splits/) using default scaling parameters (interval = c(0,10)).

Morphological variation.—The pedipalps of adult male spiders were imaged and examined for a sample representing all Appalachian species, including all major phylogroups within species (see Results). Three primary palpal features were examined as follows: shape of the median apophysis in prolateral view, shape of the conductor tip in prolateral view, and shape of the palpal tarsus in retrolateral view (see Forster et al. 1987, Figs. 39, 41). The left palp was removed and immersed in filtered 70% EtOH, and secured using KY-Jelly. Digital images were captured using a Visionary Digital BK plus system (http://www.visionarydigital.com), including a Canon 40D digital camera, Infinity Optics Long Distance Microscope, P-51 camera controller and FX2 lighting system. Individual images were combined into a composite image using Helicon Focus V5.1 software (http://www.heliconsoft.com/heliconfocus.html), which was then edited using Adobe Photoshop CS3.

RESULTS

New COI sequences (~ 900 bp) were generated for 261 individuals from 85 localities. The number of sequences collected per sampling location ranged from one to five, with an average of about three sequences per location (Table 1). All sequences can be translated to amino acids with the standard Invertebrate mitochondrial genetic code, and lack insertion/deletion characters or stop codons. Representative sequences from all sample sites, including a population set, have been deposited to GenBank (accession numbers in Table 1). Geographic location information is also available as a Google Earth KMZ file available upon request from the corresponding author.

Phylogenetic and network analysis.—RAxML searches using alternative partitioning strategies result in very similar tree topologies, with minor differences restricted to relationships between closely related sequences within terminal clades. Tree topologies resulting from different RAxML analyses have been deposited at the Interactive Tree of Life page (Letunic and Bork 2006, 2011; http://itol.embl.de/shared/mhedin). Results from the three partitions analysis are shown here (Fig. 3) and discussed below; Fig. 3 also includes bootstrap values resulting from all three partitioning strategies.

Mitochondrial gene trees support the monophyly (likelihood bootstrap > 80) of the southern Appalachian fauna, and support the monophyly of haplotypes sampled for *H. sheari, H. coylei, H. thorelli* and *H. gertschi* (Fig. 3). Monophyly is not recovered for *H. pococki*. Instead, COI sequences from this species are fragmented into five primary, geographically cohesive clades – named the "Virginia", "Elk", "Northeast", "Western" and "Central" clades (see Figs. 2, 3). Of these genetic clades, the "Virginia", "Northeast" and "Western" clades are supported (likelihood bootstrap > 80). Except for a well-supported *H. thorelli* plus *H. coylei* sister pairing, interspecific and inter-clade relationships are not supported (bootstrap < 80) in any analysis. Average K2P-corrected (Kimura 1980) pairwise genetic divergences among species and primary geographic clades are quite high, ranging from 10.6 to 15.8% (Table 3).

At shallower levels (e.g., within species and the primary geographic clades of *H. pococki*) there is considerable evidence for fractal genetic structuring. Sequence divergence among sites within species/primary clades is high, ranging from 1.9 to 14.6% (Table 3). As a point of comparison, Robinson et al. (2009) analyzed data for a taxonomically broad sample of congeneric spider species, and reported a mean K2P COI divergence between nearest interspecific neighbors (~ sister taxa) of 6.8%. Most divergences within species and geographic clades of *Hypochilus* exceed average interspecific divergence values seen in other spiders. This deep divergence within species and primary clades is geographically structured, with many well-supported, geographically cohesive nested clades

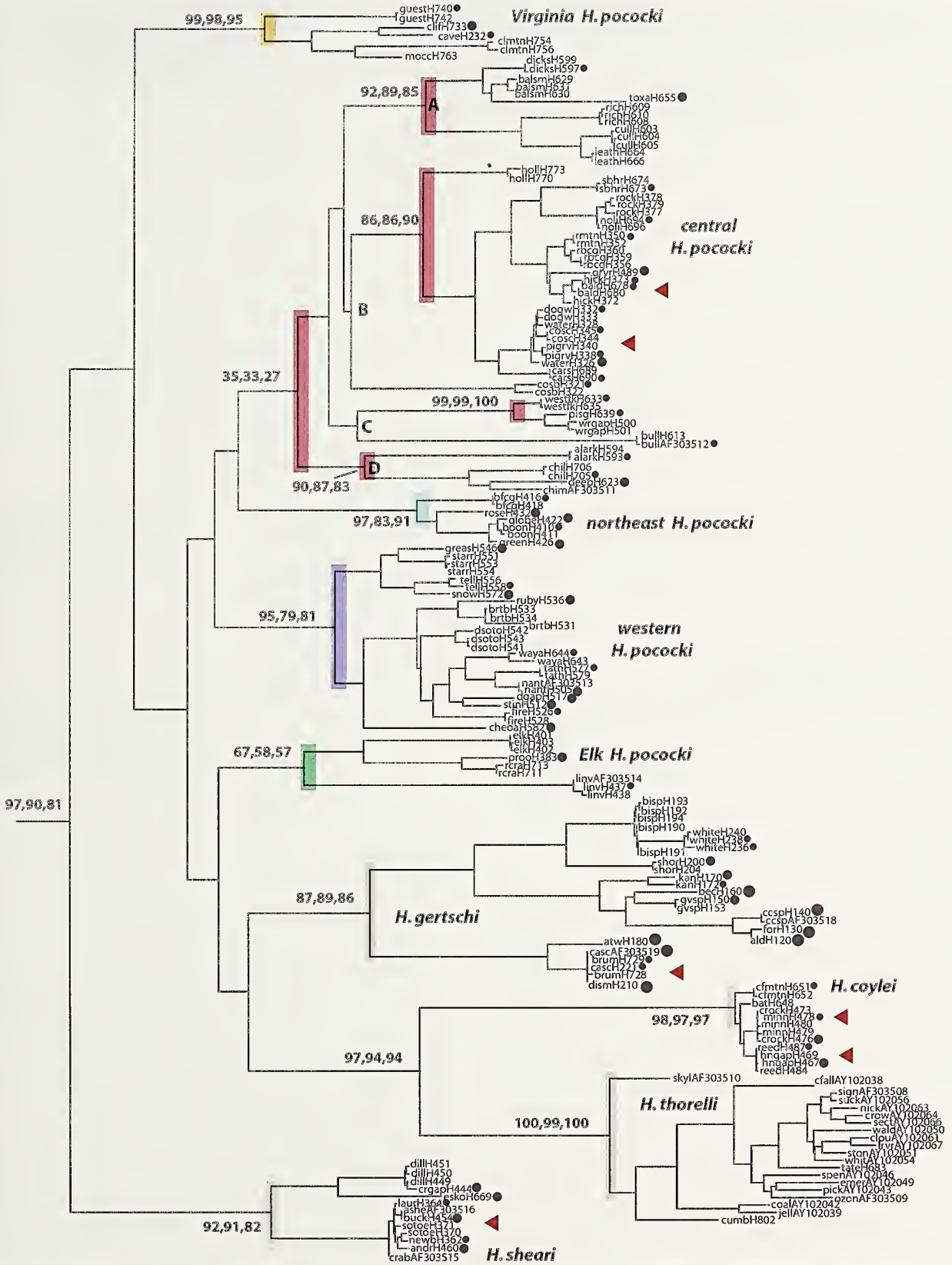

Figure 3.—ML tree reconstructed from three partitions analysis. Site acronyms are found in Table 1. Geographic clade colors for *H. pococki* correspond to those in Fig. 2. Bootstrap values resulting from no, two and three partitions analysis (respectively) shown for primary clades discussed in text. Cases of collection site non-exclusivity highlighted with red triangles. Gray circles associated with haplotype names indicate haplotypes shared by multiple specimens, with the smallest circles corresponding to $n = 2$, largest circles corresponding to $n = 4$ specimens. Node labels A–D in the "Central" *H. pococki* clade designate the four separate GMYC clusters resolved by the single threshold model.

Table 3.—Average K2P-corrected (Kimura 1980) mtDNA pairwise divergences within and between species and primary genetic clades (for *H. pococki*). A single, randomly chosen haplotype per sampled site was used; distances were computed in PAUP* version 4.0b10 (Swofford 2002).

	1	2	3	4	5	6	7	8	9	10
1. *H. thorelli*	0.0778	0.1404	0.1341	0.1137	0.1576	0.1508	0.1401	0.1132	0.1071	0.1216
2. *H. gertschi*	–	0.1150	0.137	0.138	0.1511	0.1469	0.1386	0.1269	0.1365	0.1408
3. *H. sheari*	–	–	0.0568	0.128	0.1492	0.1420	0.1271	0.1065	0.1118	0.1168
4. *H. coylei*	–	–	–	0.0190	0.1505	0.1438	0.1285	0.1151	0.1134	0.1357
5. *H. pococki*	–	–	–	–	0.1443	–	–	–	–	–
6. *H. pococki* (Central)	–	–	–	–	–	0.130	0.1367	0.1308	0.1360	0.1379
7. *H. pococki* (West)	–	–	–	–	–	–	0.094	0.1070	0.1149	0.1194
8. *H. pococki* (NE)	–	–	–	–	–	–	–	0.042	0.1157	0.1166
9. *H. pococki* (Elk)	–	–	–	–	–	–	–	–	0.146	0.1455
10. *H. pococki* (VA)	–	–	–	–	–	–	–	–	–	0.1036

(see Figs. 1–3). For example, samples of *H. sheari* are consistently separated into western and eastern subclades, samples of *H. gertschi* form three geographic subclades, samples of "Central" *H. pococki* fall into four subclades, etc.

Finally, this "clades within clades within clades" phylogenetic structuring extends to the level of local populations, where a pattern of location-specific genealogical exclusivity prevails (i.e., haplotypes from a sampling location form clades exclusive of other sampling locations). In total, we sampled two or more individuals from 81 locations, and recovered phylogenetic patterns indicative of haplotype mixing among locations in only six places on the ML tree (see Fig. 3). Of these six instances, TCS network analyses conclusively reveal haplotype sharing in only four cases, for the species *H. coylei*, *H. sheari*, and *H. gertschi* (Fig. 4).

Significant new distributional records.—Phylogenetic analyses confirm several new noteworthy distributional records for Appalachian *Hypochilus* taxa. This includes new northwestern records for *H. sheari* (esko, crgap, dill, Fig. 2; compare to Huff & Coyle 1992, fig. 12). Other significant records (compare to Forster et al. 1987, fig. 37) include the southernmost known record and a new county record for *H. gertschi* (brum, Washington County, Virginia, Fig. 1), the northeastern-most known record for *H. thorelli* (cumb, Lee County, Virginia, Fig. 1), a new county record for *H. pococki* in eastern Tennessee (clmtn, Hancock County, Tennessee, Fig. 2), and new western records for *H. pococki* in southeastern Tennessee (greas, starr, Polk County, Tennessee, Fig. 2).

Genealogical sorting index.—The 81 locations for which we sampled two or more sequences were defined as a priori labeled groups in *gsi* analyses. The average *gsi* value across all sites and species/genetic clades is relatively high (*gsi* = 0.917), with samples from only 14 locations exhibiting a *gsi* value less than 1 (Table 4). All *gsi* values are statistically significant under permutation ($P < 0.05$).

Yule-coalescent species delimitation.—A multiple thresholds model results in 54 Appalachian GMYC multiple-sequence clusters, whereas the single threshold model results in 11 Appalachian clusters. Because we view the multiple thresholds model as unrealistic (see Discussion), we prefer the single threshold model results. The eleven clusters defined by this analysis include *H. sheari*, *H. gertschi*, and the "Virginia", "Elk", "Northeast", and "Western" *H. pococki* genetic clades. The "Central" *H. pococki* clade is resolved as four separate GMYC clusters, corresponding to nodes labeled A–D on Fig. 3. The GMYC analysis collapses *H. coylei* and *H. thorelli* together into a single cluster. Although these latter two deseribed species share some male palpal features in common (e.g., shape of male conductor tip, see Catley 1994, Figs. 28, 29), they differ consistently in female spermathecal organ shape (Catley 1994, Figs. 14, 18) and have highly disjunct geographic distributions (Fig. 1).

Morphological variation.—All digital images have been deposited at Morphbank (www.morphbank.net). We imaged a single male spider from each of five different sampling locations (see Table 1) for the species *H. sheari* (Morphbank Nos. 691466–691475), *H. coylei* (Morphbank Nos. 691476–691485), *H. thorelli* (Morphbank Nos. 691496–691505) and *H. gertschi* (Morphbank Nos. 691486–691495). Examined features of male palps conformed to respective species deseriptions (Forster et al. 1987; Huff & Coyle 1992; Catley 1994), and we noted very little geographic variation within these described taxa. For *H. pococki* we examined a single male spider from 4–5 different sampling locations ($n = 22$, see Table 1) representing all primary geographic clades ("Virginia", "Elk", "Northeast", "Western" and "Central"; Morphbank Nos. 691421–691465). This sample included single males from each of the "Central" GMYC clusters. Although minor individual-level variation is evident, specimens from different primary *H. pococki* geographic clades are conserved in male palpal morphology (see www.morphbank.net, Fig. 5).

DISCUSSION

Population structure and phylogeography.—Hedin and Wood (2002) conducted in-depth population genetic analyses of *H. thorelli* based on a sampling of mitochondrial COI sequences for 85 individuals from 19 geographic sites. In this species there exists a pervasive pattern of low within-site versus high among-site mitochondrial genetic variation; i.e., most genetic variation is apportioned among, rather than within, sampled locations. Also, these authors found no COI haplotypes shared among sampling sites, despite the close geographic proximity (e.g., within 5 km) of certain sites. Based on these genetic patterns, Hedin and Wood (2002) argued for a 'fragmentation model' of extremely limited female-based gene flow, but recognized that geographic sampling at finer spatial scales eould possibly result in patterns consistent with genetic isolation by distance.

Our emphasis here was on general comparisons among taxa, not on distinguishing alternative models within a single taxon. These general comparisons reveal that mitochondrial population genetic structuring is similar among Appalachian

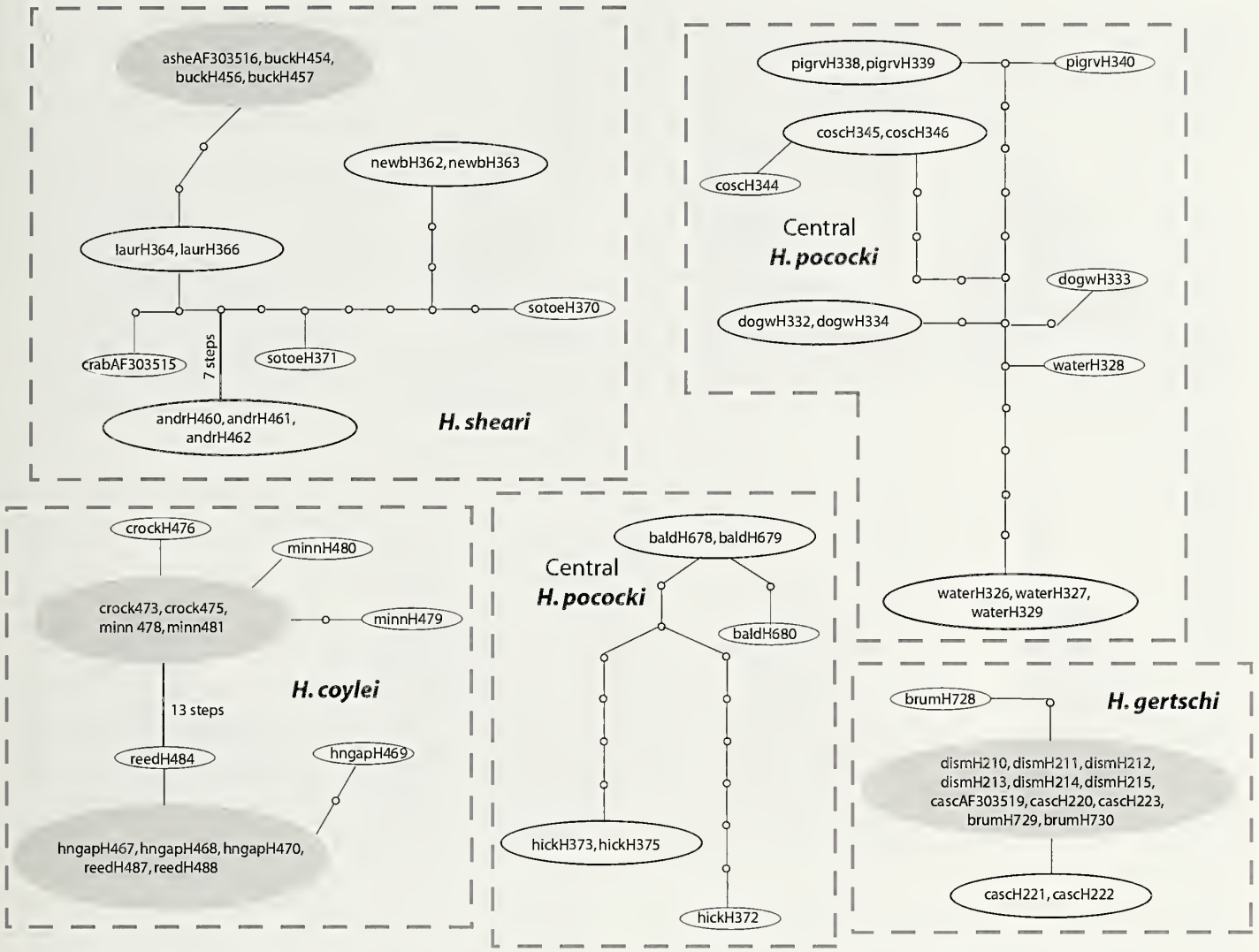

Figure 4.—TCS haplotype networks recovered at the 95% confidence level (Clement et al. 2000). Site acronyms correspond to those in Table 1.

Hypochilus species. This similarity exists despite the fact that these species are not expected to be biologically identical, and despite the fact that these species occur in different physiographic provinces of the southern Appalachians (i.e., southern Blue Ridge versus Cumberland Escarpment, etc., see Fig. 1), where we might expect rock outcrop availability and continuity to differ. For locations where we have sampled multiple specimens we find very little (if any) genetic variation, measured as the observed maximum number of nucleotide site differences per location (see Table 1). With few exceptions (see below), haplotypes from any single location form monophyletic "microclades", an inference supported by standard gene tree, network, and *gsi* analyses. Sequences in different microclades are obviously divergent, with divergence levels within phylogroups and species that are among the highest ever measured in spiders (Table 3). Overall, these patterns of mitochondrial structuring in southern Appalachian *Hypochilus* are consistent with a limited female-based gene flow scenario. This agrees with the lack of evidence for juvenile ballooning in these spiders, and with observations suggesting that the majority of adult dispersal is male-based (see Shear 1969; Fergusson 1972; Huff & Coyle 1992). This population subdivision is also consistent with many barriers to dispersal evident in the southern Appalachian Mountains.

We found a handful of instances consistent with either ongoing or historical gene flow. In both *H. coylei* and *H. sheari*, network analyses reveal identical haplotypes that are shared among sample sites (e.g., ashe & buck, crock & minn, hngap & reed – Fig. 4). Most of these cases involve locations that are relatively close in space (Fig. 2). Possible indirect evidence for gene flow is apparent for some sample locations that display high internal sequence divergence (see Table 1). For example, in "Western" *H. pococki*, haplotypes at *waya*, *brtb*, and *dsoto* are divergent (maximum divergences of 14, 19, and 16, respectively), even though these haplotypes form site-specific clades (Fig. 3). This pattern likely indicates gene flow from adjacent, but unsampled, demes. As argued in Hedin and Wood (2002), as the spatial scale of sampling more closely approximates individual dispersal distances, the pattern of zero gene flow breaks down, and the dynamic becomes more consistent with isolation by distance. The most obvious example of possible long-distance dispersal is seen in *H.*

Table 4.—GSI values.

Species	Site acronym	*gsi*	P value
H. pococki "central"	water	0.422	0.0001
"	dogw	0.664	0.0001
"	hick	0.664	0.0002
"	25 others	1	less than 0.002
H. pococki "Virginia"	4 sites	1	less than 0.002
H. pococki "Northeast"	boon	0.206	0.001
"	4 others	1	0.0001
H. pococki "western"	14 sites	1	0.0001
H. pococki "Elk"	4 sites	1	less than 0.002
H. sheari	sotoe	0.331	0.0009
"	dill	0.664	0.0001
"	6 others	1	less than 0.002
H. coylei	hngap	0.747	0.0001
"	crock	0.396	0.0003
"	minn	0.747	0.0001
"	reed	0.496	0.0001
"	cfmtn	1	0.0001
H. gertschi	brum	0.148	0.021
"	casc	0.491	0.0001
"	dism	0.491	0.0001
"	ald	0.797	0.0001
"	9 others	1	0.0001

gertschi, where identical haplotypes are shared among locations separated by large geographic distances (dism, casc, brum; Figs. 1, 4). Because northern populations of *H. gertschi* are genetically variable (Fig. 3), this may indicate population expansion toward the south from northern refugia.

Individual spiders and local populations of Appalachian *Hypochilus* species are almost always restricted to sheltered rock outcrop habitats (Hoffman 1963; Fergusson 1972; Forster et al. 1987; Huff & Coyle 1992; this study). As such, dispersal barriers must somehow coincide with areas where such habitat is lacking, although there are also instances where spiders are apparently lacking from seemingly suitable rocky habitat (e.g., see Huff & Coyle 1992, fig. 12), likely because of unsuitability of more general environmental factors (e.g., elevation, temperature, humidity, etc.). We suggest that future studies combine much denser geographic sampling with formal ecological niche modeling to understand how landscape factors impact the distribution of genetic variation in these spiders (i.e., landscape genetics, see Storfer et al. 2010).

Species delimitation in appalachian *Hypochilus*.—*Hypochilus* spiders possess a suite of shared biological characteristics consistent with what we term the "cryophilic syndrome". Commonalities of this syndrome include a restriction to specialized microhabitats that are naturally spatially fragmented (e.g., sheltered rock outcrops in mesic situations, etc.). Limited dispersal abilities, in combination with habitat specialization, result in pervasive population genetic subdivision and the evolution of divergent genetic groupings. Over longer evolutionary timescales, limited dispersal abilities result in many species that are geographically confined to small areas (short-range endemic taxa, sensu Harvey 2002; e.g., *H. coylei* and *H. gertschi*). In arrays of parapatric short-range endemic taxa, species syntopy is rare, probably because of ecological niche conservatism that prevents resource partitioning; this ecological niche conservatism likely plays an important role in speciation (following model of Wiens 2004). Finally, "cryophilic syndrome" taxa are also often morphologically conserved, perhaps reflecting stabilizing selection on morphology because of ecological niche conservatism. The combination of extreme population genetic subdivision with functional (i.e., ecological and morphological) conservatism implies that divergent genetic groupings often lack obvious functional divergence, or show only subtle functional divergence.

We are most familiar with taxa exhibiting the "cryophilic syndrome" in arachnids and other arthropods, although some vertebrate taxa also share these features (e.g., *Batrachoceps* salamanders, Joekusch & Wake 2002; Wake 2006; *Xantusia* night lizards, Sinclair et al. 2004; Leavitt et al. 2007). In arachnids, integrative studies assessing both genetic and functional divergence have revealed patterns consistent with this syndrome in many small-bodied harvestmen taxa (e.g., Boyer et al. 2007; Thomas & Hedin 2008; Hedin & Thomas 2010; Schönhofer & Martens 2010). Ground-dwelling mygalomorph spiders are also conspicuous in this regard (Bond et al. 2001; Hendrixson & Bond 2005; Arnedo & Ferrandez 2007; Starrett & Hedin 2007; Bond & Stockman 2008).

When divergent genetic groupings lack obvious functional divergence, the process of species delimitation is very challenging, and must incorporate multiple lines of evidence. This is indeed the case for southern Appalachian *Hypochilus*. The interpretation of contrasting data patterns is difficult, with genetic data suggesting high divergence and many separate lineages, whereas functional data suggest limited divergence and fewer distinct lineages. This contrast provides interesting insight into how these lineages evolve, but what are the species limits? A "many cryptic species" hypothesis would include as distinct species four named *Hypochilus* taxa (*H. sheari, H. coylei, H. thorelli, H. gertschi*), plus divergent phylogroups within *H. pococki*. Under the GMYC single threshold model, four additional species would be resolved within "Central" *H. pococki*. It is important to note that all of these genetic groups possess qualities consistent with species status under many different species criteria (see Sites & Marshall 2004), including reciprocal monophyly, high interspecific divergence, and contiguous geographic distributions (Figs. 2, 3). Also, a geographic pattern of several species with relatively small and allopatric distributions is expected for organisms with low vagility, particularly in a region as topographically complex as the southern Appalachians.

However, there are several problems with this "many cryptic species" interpretation. First, because mtDNA reflects only maternal genetic histories, it is not known whether observed population genetic structuring extends to both genomes. Is male-based gene flow in these spiders extensive enough to act as a cohesive evolutionary force? Second, theory demonstrates that deep mitochondrial genealogical breaks can arise stochastically in low dispersal systems (Irwin 2002; Kuo & Avise 2005), again making it difficult to interpret the significance of observed genetic patterns. Finally, even if the genetic system used here was biparental, fractal genetic structuring makes it difficult to define boundaries of higher-

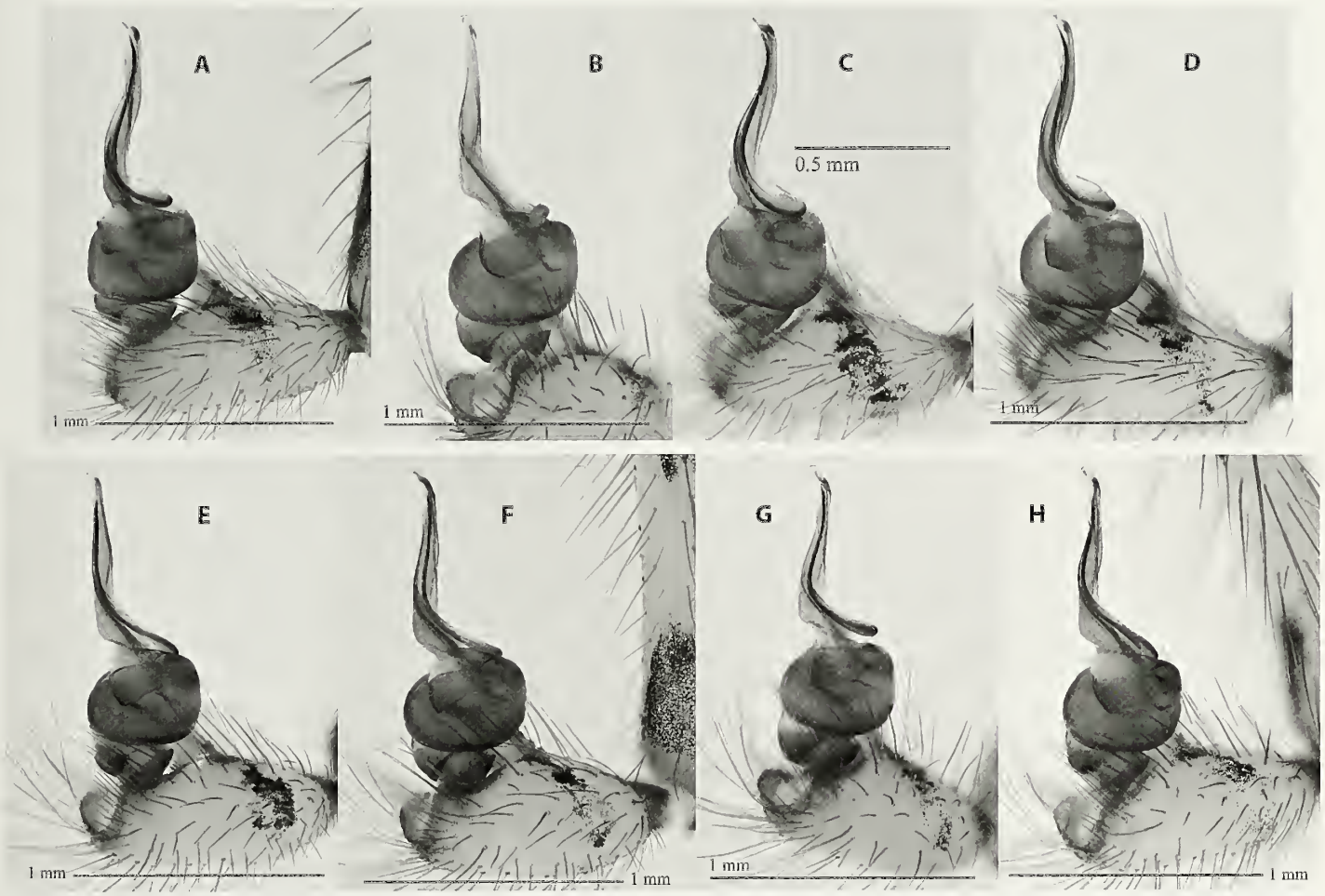

Figure 5.—Representative variation in male palpal morphology in *H. pococki*: A) "Central" Clade, GMYC cluster A, Toxaway; B) "Central" Clade, GMYC cluster B, Dogwood Flats; C) "Central" Clade, GMYC cluster C, West Fork Pigeon River; D) "Central" Clade, GMYC cluster D, Chilhowee; E) "Elk" Clade, Elk River; F) "Northeast" Clade, Green Mtn.; G) "Virginia" Clade, Cliff Mtn.; H) "Western" Clade, Greasy Creek. All views prolateral.

level units, e.g., phylogeographic units versus species, because genealogical breaks are ubiquitous. Some authors have argued that significant intraspecific population structure may confound GMYC analyses (Lohse 2009; but see Papadopoulou et al. 2009), and we reject the multiple thresholds GMYC model (implying 54 species) for this reason.

In light of the potential limitations of mitochondrial gene tree data discussed above, we favor a more conservative perspective (based on male genitalic morphology in particular), and do not recommend taxonomic changes at this time. This conservative, functional divergence perspective treats different named species as distinct, as these taxa differ in genital morphology. This interpretation is not without difficulties. First, we must accept the genetic non-monophyly of a species-level taxon (i.e., *H. pococki*), although it could be argued that this non-monophyly reflects inaccurate gene tree estimation (e.g., due to mutational saturation, etc.). Second, if we accept the premise that separate species can be morphologically cryptic (at least as considered with current technology; see Saez & Lozano 2005; Bickford et al. 2007; Daniels et al. 2009), then it is clearly possible that a conservative perspective potentially undersplits Appalachian *Hypochilus* species diversity. To further test species delimitation hypotheses in this challenging group we recommend a multigenic genealogical approach. This would include the collection of DNA sequence data from many independent nuclear markers, clearly feasible given the increase in genomics tools (e.g., via next-generation sequencing) for non-model systems (e.g., see Thomson et al. 2010). Such data could then be combined with new methods for species delineation (Yang & Rannala 2010; Leaché & Fujita 2010) to delimit species as groups that represent genetic clades recovered for multiple loci, with or without functional diagnosability. The research presented here pinpoints geographic regions and potential cryptic lineages to target under such a study plan.

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Nearctic species of the new genus *Tigrosa* (Araneae: Lycosidae)

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Abstract. The new genus *Tigrosa* is established for five Nearctic species originally described in the genus *Lycosa* Latreille 1804. Four of the species are transferred from *Hogna* Simon 1885: *H. annexa* (Chamberlin & Ivie 1944), *H. aspersa,* (Hentz 1844), *H. grandis* (Banks 1894) and *H. helluo* (Walckenaer 1837). The remaining species, *Allocosa georgicola* (Walckenaer 1837) is transferred from *Allocosa* Banks 1900. The presumed synapomorphy that supports *Tigrosa* is the color pattern on the dorsum of the cephalothorax, which is described and illustrated. In addition to their distinct color pattern, *Tigrosa* species are very similar in characteristics of the male palpus and epigynum, details of the eye arrangement, leg length in relation to body dimensions, as well as foraging habits. Comparisons made between *Hogna,* as defined by the type species *H. radiata,* Latreille 1817, and *Tigrosa,* as defined in this paper, demonstrate distinct differences in dorsal color pattern, structure of the epigynum, dimensions of the eye rows, color pattern of the venter and habitat preferences.

Keywords: *Lycosa*, *Hogna*, systematics, nomenclature, zoogeography

This is the fourth paper in a projected series of systematic studies of the Nearctic Lycosidae, formerly described in the genus *Lycosa* Latreille 1804. Over 50 species of medium to large wolf spiders from the Nearctic region were previously placed in this genus. Dondale & Redner (1990) pointed out that "none of the North American species belong to the genus *Lycosa* (in the restricted European sense)." According to Zyuzin & Logunov (2000) the genus *Lycosa* should be restricted to a group of large burrowing wolf spiders in the Mediterranean region; therefore, the genus *Lycosa* does not occur in North America. Dondale & Redner (1990) placed seven Canadian species, previously recognized as *Lycosa,* in the genus *Hogna* Simon 1885. In that paper they indicated that *Hogna* might eventually need to be separated into two or more genera. *Hogna rabida* (Walckenaer 1837) and *Hogna punctulata* (Hentz 1844), together with three other species originally described in *Lycosa,* were assigned by Brady & McKinley (1994) to *Rabidosa* Roewer 1954. The dorsal color pattern of the cephalothorax and abdomen was the presumed synapomorphy that connected these five species and separated them from other large lycosids. A number of shared characteristics described by Brady & McKinley (1994) also served as a basis for the recognition of *Rabidosa* as a distinct genus.

Since the publication by Dondale & Redner (1990), many of the large lycosids formerly described in the genus *Lycosa* have been considered to belong to the genus *Hogna.* Because of the paramount position of the Mediterranean *Lycosa radiata* Latreille 1817 as the type species of the genus *Hogna,* a clear definition and description of this species is critical to understanding the relationship of this species to many large wolf spiders in North America. Dondale & Redner (1990) distinguished *Hogna* from other genera of lycosids by the following characteristics: "carapace uniform in height; cymbium with 2 or more terminal macrosetae; embolus with large arch at base; terminal apophysis sickle-shaped and often double; and median apophysis with spur at base." Thus, the genus *Hogna* is diagnosed chiefly by characteristics of the male palpus. In the past 25 years of my study of large Lycosidae, primarily from North America, but also including European, Central American, South American and Australian species, I have found that the palpal characteristics used by Dondale & Redner (1990) to define *Hogna* are world wide in distribution and occur in species that are distinctly different in other aspects of their morphology and biology. The structure of the palpus in many large lycosids previously described in *Lycosa* is structurally conservative and consequently plesiomorphic within the subfamily Lycosinae. Therefore, additional morphological characteristics as well as other biological and zoogeographical features need to be examined in order to determine evolutionary relationships and define genera within the Lycosidae.

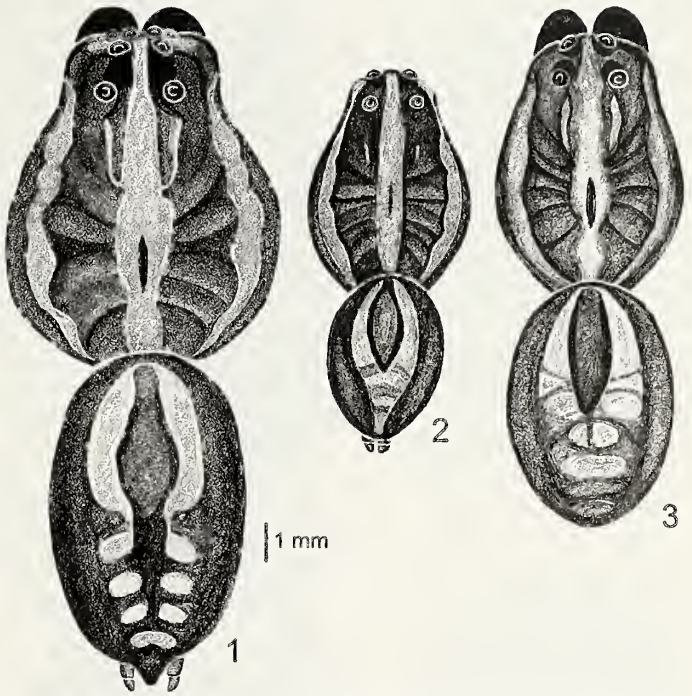
Because of their importance as the type species of *Hogna*, in 2006 I requested specimens of *Lycosa radiata* from the Natural History Museum in London and the Muséum national d'Histoire naturelle in Paris. After carefully examining and drawing a number of these specimens identified as *Lycosa radiata* Latreille, it became apparent that at least two different species were represented in these collections. Ultimately I carefully studied and decided upon ten females and ten males from localities in Western Europe, primarily from France and the Island of Minorca, as representatives of *H. radiata.* These specimens were used for descriptions, measurements and illustrations to present a morphological view of the Mediterranean genus *Hogna* in order to compare it to the North American genus *Tigrosa.* Specimens from Africa and other Mediterranean localities that clearly represented a different species were rejected as examples of *H. radiata.* From my study of the above museum collections, it became very doubtful in my mind that the geographical range of *H. radiata* should include central African specimens, and perhaps even those from central Asia. A thorough study of European, Asian and African specimens now considered as *Hogna radiata,* should definitely reveal at least two distinct species, and probably more. It is not the purpose of this paper to solve this systematic problem, but simply to distinguish the genus *Tigrosa* from *Hogna.* For that purpose 20 specimens identified as *Hogna radiata* from the British Museum of Natural History and the Muséum national d'Histoire naturelle were used to provide a systematic account of this species. It became apparent from this investigation that *Hogna radiata* is not closely related to the species in North America described here as the new genus *Tigrosa.*

The new genus *Tigrosa* embraces five species: *Hogna annexa* (Chamberlin & Ivie 1944), *Hogna aspersa* (Hentz 1844), *Allocosa georgicola* (Walckenaer 1837), *Hogna helluo* (Walckenaer 1837) and *Hogna grandis* (Banks 1894), all previously assigned to *Lycosa*. The primary characteristic uniting these five species and distinguishing them from other species groups formerly described in *Lycosa* and *Hogna* is the dorsal color pattern on the cephalothorax. In addition these five species are alike in characteristics of the female genitalia and male palpus, details of the eye arrangement, color pattern of the venter, body structure (including the ratio of leg length to carapace width) and foraging habits. Except for certain features of the palpal structure, all of these characteristics clearly separate the new genus *Tigrosa* from *Hogna*.

Because of their widespread distribution and geographic variation as well as individual variation within sympatric populations, the genus *Tigrosa* has proven to be a challenge for various investigators. *Tigrosa annexa* and *T. georgicola* were unknown before Chamberlin & Ivie's (1944) paper on the spiders of the Georgia region. Even in later publications there was considerable confusion about the taxonomic identity of the five species representing the genus *Tigrosa*. Although only five species are recognized in this study, the number of specimens represents about 20% of all the thousands of large lycosids examined during the past 25 years. Various investigations of these large lycosids have been hampered by the lack of an understanding of their systematic relationships. One of the primary goals of this investigation has been to clarify these relationships. Another important consideration was to stabilize the nomenclature of the species described here under *Tigrosa*, and to provide illustrations and a key that would allow their correct identification by lay persons and arachnologists interested in ecology and behavior rather than systematics per se.

METHODS

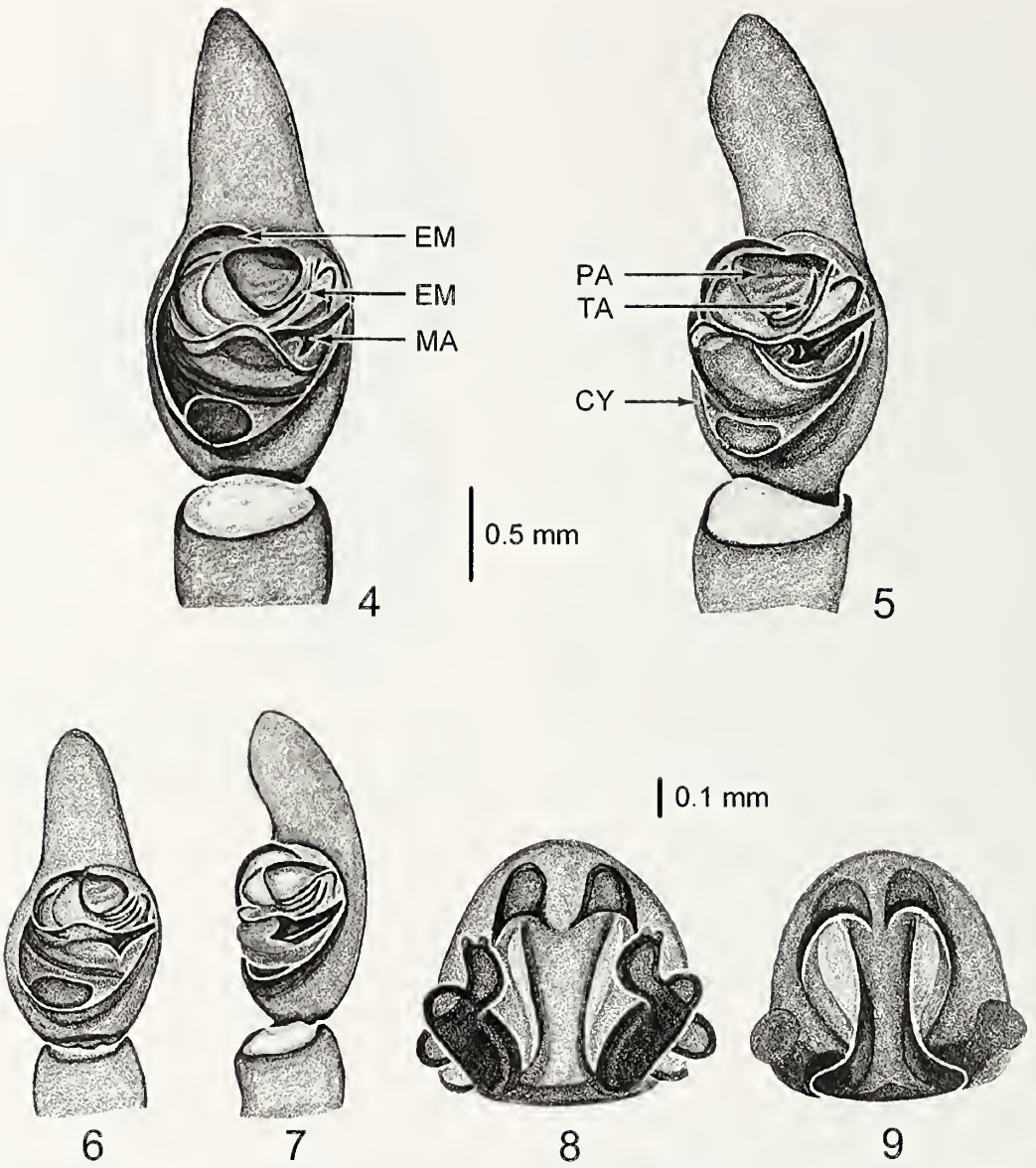
All measurements are in millimeters. Scales for drawings are provided for each illustration. The scales for dorsal views represent 1 mm (Fig. 1), the scales for views of the male palpi represent 0.5 mm (Fig. 4), and the scales for views of the female genitalia represent 0.1 mm (Fig. 8). A net micrometer (1.0 mm) was used in an ocular lens (8×) with a combination of low (1×) and high (4×) power objectives for making measurements. The higher power combination was used to measure the eye rows and was determined to be accurate to 0.2 units of the micrometer grid or 0.066 mm. Therefore, in comparing dimensions of eye rows, the eye rows are considered subequal if they are less than 0.07 mm different. The lower power combination was used in measuring the body dimensions and leg segment lengths and was determined to be accurate to 0.2 units of the micrometer grid or 0.266 mm. Therefore, in comparing body dimensions and leg segment lengths, these structures are considered subequal if they are less than 0.27 mm different. Brady (1979) described in detail the rationale and procedures for measurements and illustrations. Color descriptions and illustrations are based upon observations of specimens preserved and submerged in 75–80% ethanol under low power of a Leitz dissecting microscope. Illumination was provided by a Reichert microscope light. Tibial spination has been found to show little variation within the same sex of congeneric species of large North American lycosid genera that I have examined, and therefore has not been noted in this paper.



Figures 1–3.—Dorsal view of *Tigrosa annexa*: 1. Large male from Washington County Mississippi; 2. Small male from 1 mi. [1.6 km] E of Union, Newton County, Mississippi; 3. Female from 2 mi. [3.2 km] N of Stoneville, Washington County, Mississippi.

Geographic localities.—In order for investigators to navigate distribution records under Species Examined, I have tried to present geographic localities as accurately and concisely as possible. States of North America are listed from North to South and East to West. There are three different types of entries under States exemplified by the following: 1) Single record: Ottawa Co., Grand Haven (43.00°N, 86.23°W), 30 September 1968, W. Defeyter, HCC, 1♀; 2) Multiple records, same locality, same collector: Emmet Co., Bayview (45.39°N, 84.93°W), 21 July 1937, 1♀, 14 July 1938, 1♀, 10 July 1941, A.M. Chickering, MCZ, 1♀, and 3) County only: Washtenaw Co. (42.25°N, 83.84°W) A.M. Chickering, MCZ, 1♀. The geographical coordinates listed for Counties only are those of the county seat.

Abbreviations.—*Collections:* AMNH = American Museum of Natural History, New York; BMNH = The Natural History Museum, London; DMNS = Denver Museum of Nature and Science, Denver, Colorado; FSCA = Florida State Collection of Arthropods, Gainesville; HCC = Hope College Collection, Holland, Michigan; MCZ = Museum of Comparative Zoology, Harvard University, Cambridge, Massachusetts; MNHP = Muséum national d'Histoire naturelle, Paris; MSST = Mississippi State University Collection, Starkville, Mississippi; MWSU = Midwestern State University, Wichita Falls, Texas. *Morphological*: Eye Arrangement: AME = anterior eyes, ALE = anterior lateral eyes, PME = posterior median eyes, PLE = posterior lateral eyes, POQ = posterior ocular quadrangle. Male: CY = cymbium; EM =



Figures 4–9.—*Tigrosa annexa*: 4, 5. Large male from Washington County, Mississippi; 4. Left palpus, ventral view; 5. Left palpus, retrolateral view. 6, 7. Small male from 1 mi. [1.6 km] E of Union, Newton County, Mississippi; 6. Left palpus, ventral view; 7. Left palpus, retrolateral view. 8, 9. Female from Washington County, Mississippi; 8. Vulva, dorsal view; 9. Epigynum, ventral view.

embolus; MA = median apophysis; PA = palea; TA = terminal apophysis. Female: MS = median septum; LP = longitudinal piece; SP = spermathecae, TP = transverse piece. *Records*: The lower case "i" is used to indicate immature specimens.

SYSTEMATICS

Tigrosa new genus

Lycosa Walckenaer 1837:337, 338 (part); Hentz 1844:389; Blackwall 1846: 30; Emerton 1885:482, 487; Stone 1890:423; Banks 1892:66–68, 1894:49; Tullgren 1901:18; Emerton 1902:59; Montgomery 1902:557, 559; 1904:290; Chamberlin 1904:286, 1908:26, 234, 236; Comstock 1913:633, 1940:645; Gertsch 1934:6; Muma 1943:46; Wallace 1950:74; Roewer 1955:210; Bonnet 1957:2621; Griswold 1993:3.

Tarentula Blackwall 1846:30 (part); C.L. Koch 1847:135; Simon 1864:350; Keyserling 1877:634; McCook 1879:xi.

Leimonia Simon 1864:351 (part).

Trochosa Keyserling 1877:659 (part).

Allocosa, Roewer 1955:210 (part).

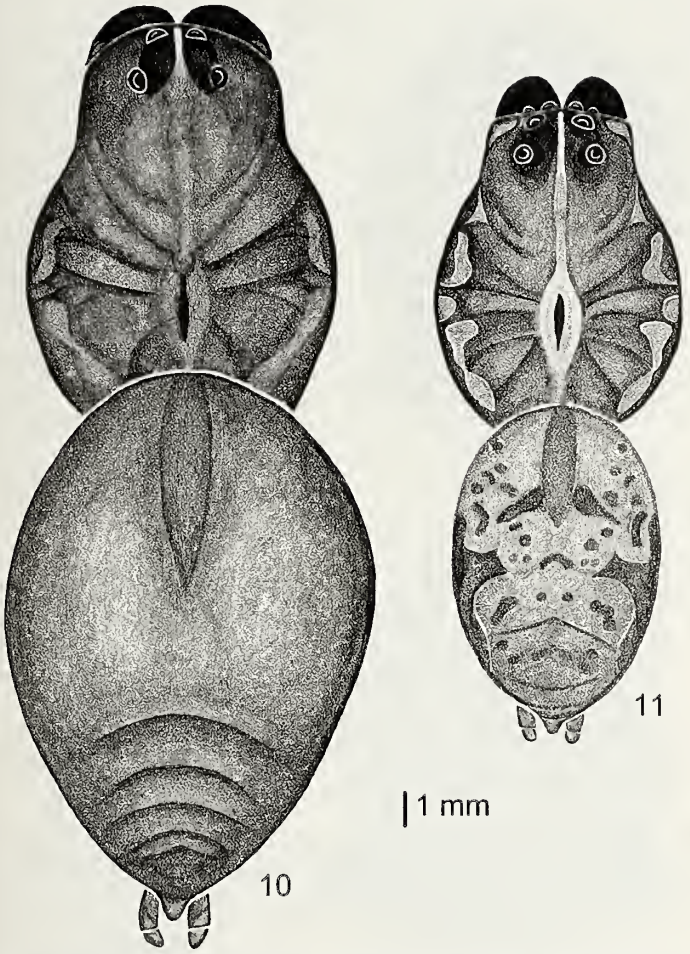
Geolycosa Roewer 1955:244 (part).

Hygrolycosa, Roewer 1955:261 (part).

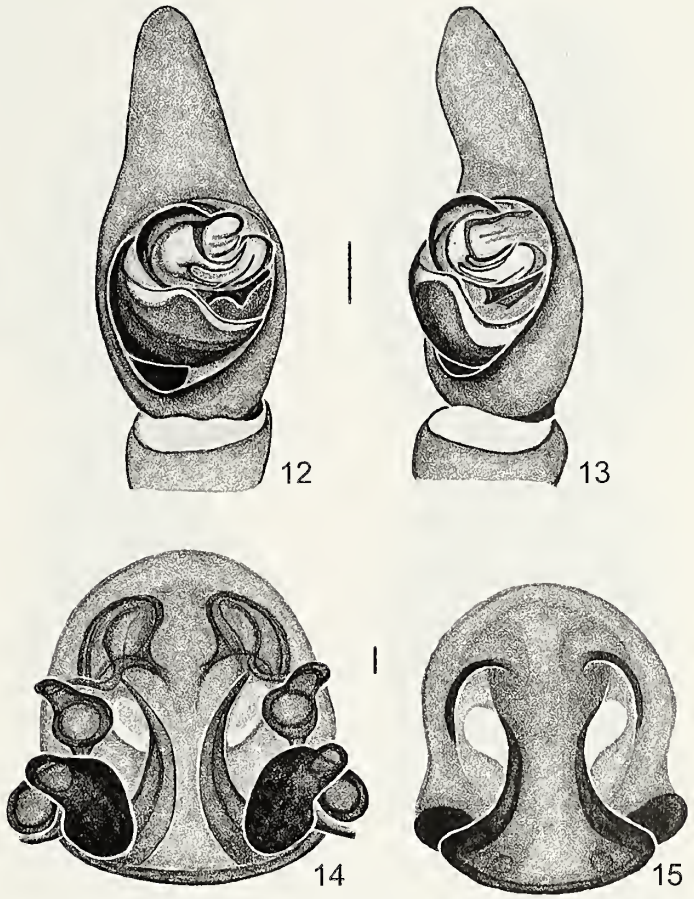
Hogna Roewer 1955:358, 359 (part); Dondale & Redner 1990:49–51; Bennet 1992:42–43; Paquin & Duperre 2003:161; Slowik & Cushing 2009:261; Platnick 2011.

Type species.—*Tigrosa helluo* (Walckenaer 1837)

Etymology.—The generic name is derived from the iconic mammal, the tiger, because of the fierce nature of the species found in *Tigrosa* and in recognition of the stripes on the dorsal surface of the body and contrasting dark and light markings on the legs of most species. According to Don Cameron (2005) the genus name *Lycosa* is the feminine singular present participle of a verb meaning "fierce like a



Figures 10–11.—Dorsal view of *Tigrosa aspersa*: 10. Female from Imboden, Lawrence County, Arkansas; 11. Male from Imboden, Lawrence County, Arkansas.

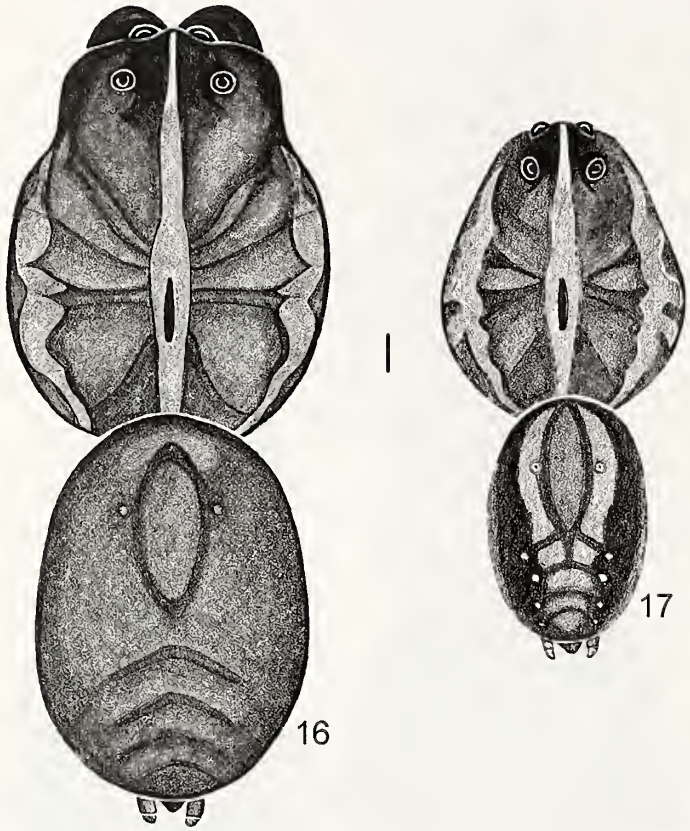


Figures 12–15.—*Tigrosa aspersa*: 12, 13. Male from Imboden, Lawrence County, Arkansas; 12. Left palpus, ventral view; 13. Left palpus, retrolateral view. 14, 15. Female from Imboden, Lawrence County, Arkansas: 14. Vulva, dorsal view; 15. Epigynum, ventral view. Scale bars: palpi, 0.5 mm; epigyna, 0.1 mm.

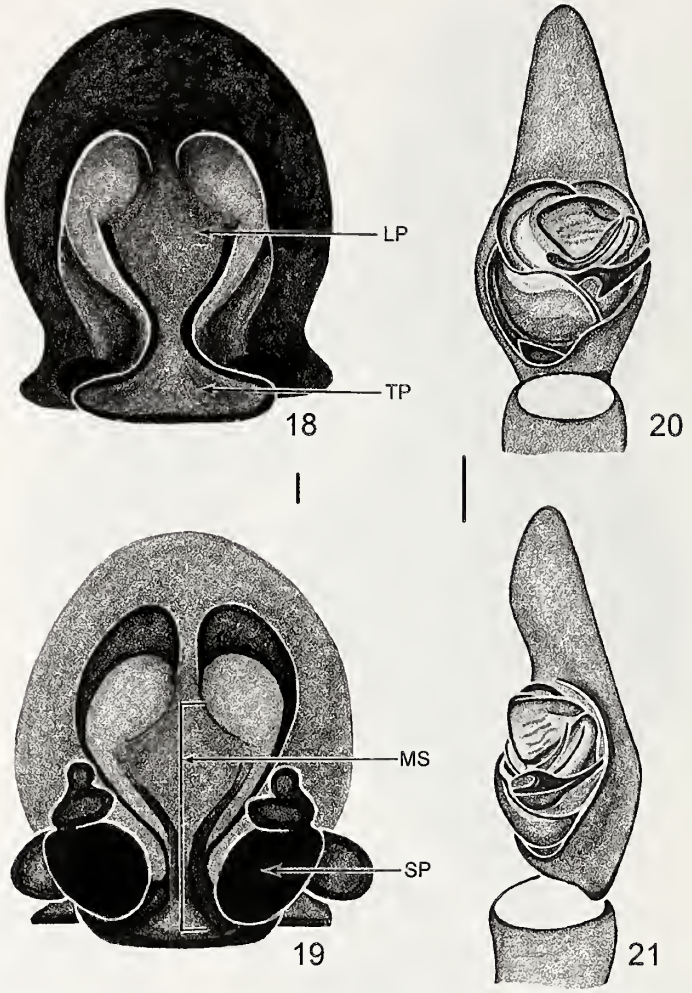
wolf"; therefore, *Tigrosa* can be freely translated as "fierce like a tiger."

Diagnosis.—*Tigrosa* is distinguished from other large lycosids by the dorsal color pattern on the carapace that consists of a narrow pale cream to yellow median stripe that extends posteriorly from the AME row to the posterior declivity of the cephalothorax, except in the female of *T. aspersa* where it is limited to the eye region. The median stripe throughout its length is not wider than the space between the PME. In addition broad irregular or scalloped submarginal stripes extend from the posterior cephalic region of the carapace to the posterior declivity. A darker background color on the carapace ranging from light yellowish brown to dark reddish brown provides contrast to the lighter stripes. In addition, distinct black lines radiate from the cephalic groove to the lighter submarginal stripes. The dorsal pattern is best visualized by reference to the illustrations in Figs. 1–3, 10, 11, 16, 17, 22, 23, 28 and 29, which exemplify this synapomorphic feature. *Tigrosa* is also characterized by an inverse T-shaped epigynum, a feature of many lycosine genitalia (Figs. 9, 15, 18, 26, 33) and similar components of the internal female genitalia (Figs. 8, 14, 19, 27, 32). Males of *Tigrosa* all have a well-developed median apophysis with a ventrally directed point or spur. They also exhibit a two-part terminal apophysis, found in many lycosine pedipalps, and similar palea shape (Figs. 6, 7, 12, 13, 20, 21, 24, 25, 30, 31). Species of *Tigrosa* are alike in eye arrangement and are all robust, with stout bodies and long legs (see Tables 1–5). *Tigrosa* differs from *H. radiata*, the type species of *Hogna,* in dorsal color pattern (compare Figs. 1, 10, 16, 22, 28 with Fig. 34), color pattern on the venter (compare Figs. 40–44 with Fig. 45), and certain dimensions of the eye row (compare Tables 1–5 with Table 6).

Remarks.—A brief comparison of *Tigrosa* and *Hogna* is presented here to emphasize differences between these genera. More detailed descriptions of *Hogna* and *Hogna radiata* appear under the diagnosis of *Hogna* later in this paper. In *Tigrosa* the presumed synapomorphy that connects its members is the dorsal pattern on the cephalothorax. It is characterized by a narrow cream to yellow median stripe on the carapaee that begins in the AME region and continues to the posterior declivity. This stripe widens in the thoracic area, but its width throughout its length does not exceed the space between the PME (Figs. 28, 29). In *H. radiata* the pale yellow median stripe is much wider, and its width exceeds the space



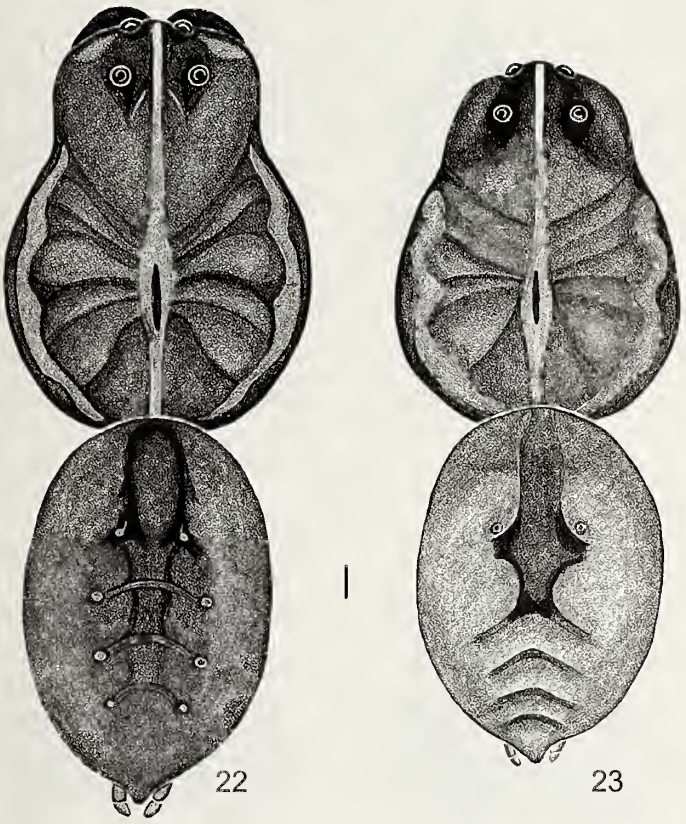
Figures 16, 17.—Dorsal view of *Tigrosa georgicola*: 16. Female from 2 mi. [3.2 km] N of Stoneville, Washington County, Mississippi; 17. Male from 2 mi. [3.2 km] N of Stoneville, Washington County, Mississippi. Scale bar, 1 mm.



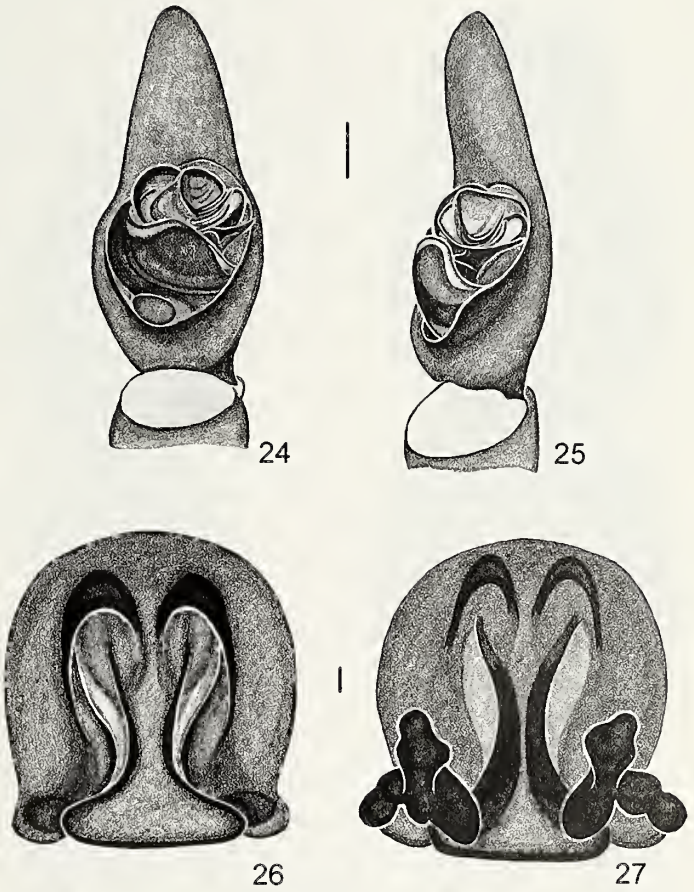
Figures 18–21.—*Tigrosa georgicola*: 18, 19. Female from 2 mi. [3.2 km] N of Stoneville, Washington County, Mississippi. 18. Epigynum, ventral view; 19. Vulva, dorsal view. 20, 21. Male from 2 mi. [3.2 km] N of Stoneville, Washington County, Mississippi. 20. Left palpus, ventral view; 21. Left palpus, retrolateral view. Scale bars: palpi, 0.5 mm; epigyna, 0.1 mm.

between the PME (Figs. 34, 35). *T. helluo* has narrow yellow to yellow-brown submarginal stripes beginning behind the PME row and continuing posteriorly (Figs. 28, 29). In *H. radiata* the pale submarginal stripes begin in the vertical facial area, are broader, and often reach to the margins of the carapace (Figs. 34, 35). The cardiac mark on the dorsum of the abdomen in *T. helluo* is often outlined in dark brown or black (Figs. 28, 29). In *H. radiata* (Figs. 34, 35) the cardiac mark is also outlined in black but includes two distinct black dots along the posterior margin, a condition not found in *Tigrosa*. The ventral surface of the abdomen posterior to the epigastric furrow in *H. radiata* is entirely black (Fig. 45). In *Tigrosa* the venter of the abdomen is usually cream to light brown in overall color with scattered black spots (Figs. 40, 41), without conspicuous black dots or dashes in the central area (Fig. 43), or with spots or dashes arranged in longitudinal rows (Figs. 42, 44). Fundamental differences also occur between *Hogna* and *Tigrosa* in the eye arrangement. For example, the anterior eye row width in *Tigrosa* is subequal to the PME row width (0.17 mm or less difference), but in *Hogna radiata* the anterior eye row width is obviously less than the width of the PME row (0.30 mm or more difference). Also the length of the POQ in *Tigrosa* (with the exception of *T. aspersa*) is equal to the width of the anterior eye row (0.02 mm or less difference), but in *Hogna radiata* the POQ length is greater than the width of the anterior eye row (0.14 mm or more difference). In other words, the eyes in *Tigrosa* are spaced in a different geometric configuration than in *Hogna*. In the epigynum of *H. radiata* (Fig. 39) the LP is longer in proportion to the TP than in *Tigrosa* (Figs. 9, 15, 18, 26, 33), and unlike *Tigrosa*, the sides of the LP are parallel, and there are lateral grooves along the length of the LP.

Description.—Total body length of 100 specimens measured (rounded to mm): females: 10 to 31 mm, males: 11–24 mm; carapace length: females: 6–13 mm, males 6–12 mm; carapace width: females 4–10 mm, males: 4–9 mm. Carapace viewed dorsally: AME row smoothly convex along lateral margins, with posterior margin concave; viewed laterally: essentially the same height from eye region to posterior declivity, highest point at posterior cephalic region in front of dorsal groove with the carapace sloping very slightly anteriorly. Dorsal groove long and distinct. Dorsal color pattern with narrow pale cream to yellow median stripe from PME row to posterior edge of cephalothorax. The pale submarginal stripes have uneven sides with edges sealloped or indented. Lighter stripes surrounded by darker brown to very

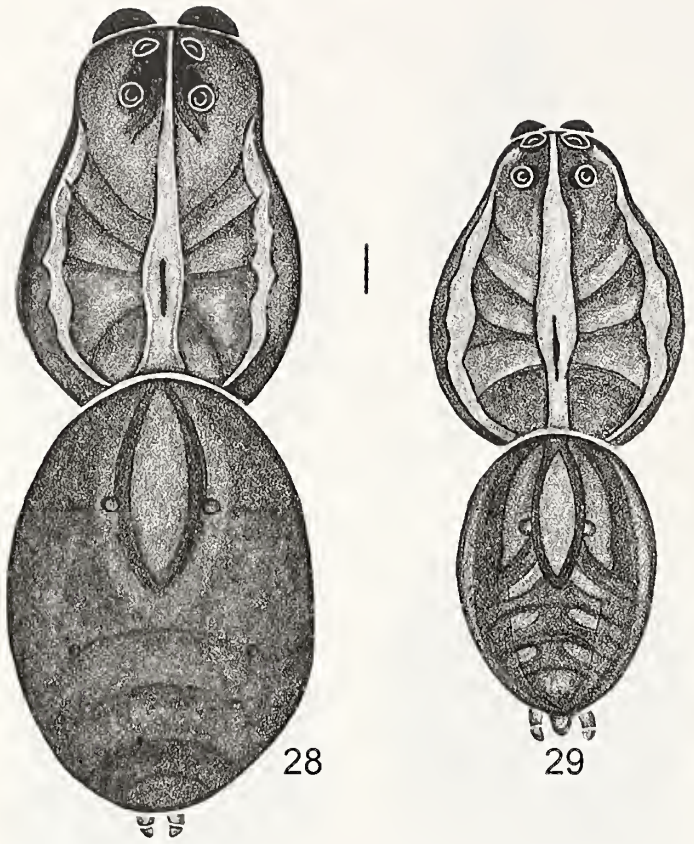


Figures 22, 23.—Dorsal view of *Tigrosa grandis*: 22. Female from Ft. Collins, Larimer County, Colorado; 23. Male from Ft. Collins, Larimer County, Colorado. Scale bar, 1 mm.

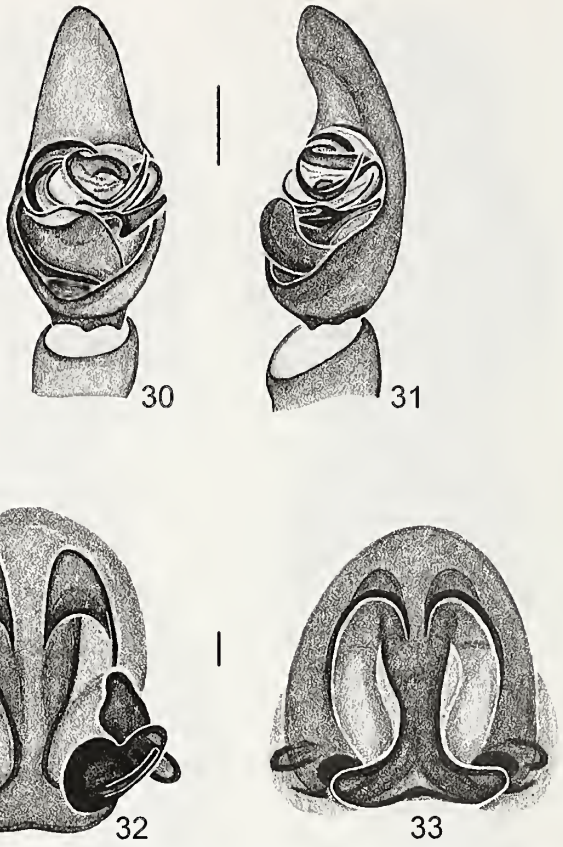


Figures 24–27.—*Tigrosa grandis*: 24, 25. Male from Ft. Collins, Larimer County, Colorado. 24: Left palpus, ventral view; 25. Left palpus, retrolateral view. 26, 27. Female from Ft. Collins, Larimer County, Colorado. 26. Epigynum, ventral view, 27. Vulva, dorsal view. Scale bars: palpi, 0.5 mm; epigyna, 0.1 mm.

dark reddish brown color. AME slightly larger than ALE. AME eye row subequal to PME row. PLE row much the widest. See Tables 1–5 for more precise measurements of selected population samples. Chelicerae dark reddish brown to black; anterior and posterior margins each with three teeth. Posterior teeth are approximately the same size. In the anterior row the central tooth is largest and is adjoined by a very small inside tooth (nearer the midline) and a smaller outside tooth (farthest away from the midline). Background coloration ranges from pale yellow, yellow-orange to brown with distinct irregular dark brown to black bands or annulations that are usually found in *Tigrosa aspera, T. georgicola, T. helluo,* and *T. grandis,* but are absent in *T. annexa.* Order of leg length from longest to shortest is IV-I-II-III. In *Tigrosa* dorsal abdominal and ventral color and patterns are much more highly variable than the color and pattern on the cephalothorax. Features of the abdomen are influeneed more by physiological condition (e.g., gravid or starved individuals), hirsuteness of individuals and habitat. Representative patterns were chosen for illustrations of the venter in Figs. 40–44. Dorsum of abdomen generally with a dark cardiac mark, often lanceolate, and outlined in darker color in the midsection. This is bounded laterally by lighter color ranging from cream to light brown created by an admixture of pigment and tufts of hair creating a mottled appearance (Figs. 1–3). The venter of the abdomen ranges from cream to light brown with darker brown spots as in *T. annexa* (Fig. 40) and *T. helluo* (Fig. 41), coalescing spots forming longitudinal rows as in *T. georgicola* (Fig. 42) and *T. aspersa* (Fig. 44), or mottled as in *T. grandis* (Fig. 43). Male palpus with stridulatory file situated retrolaterally at tip of tibia. Cymbium (CY in Fig. 4) with cluster of macrosetae at tip and with stridulatory scraper retrolaterally at base. Male palpal sclerites as seen in ventral view of left palpus: palea (PA in Fig. 5) retrolateral in position, moderately developed and weakly sclerotized; embolus (EM in Fig. 4) originating behind palea, broadly curving in prolateral area of cymbium in a counter clockwise direction, then adjoining the lower sickle-shaped tip (often difficult to see) of the terminal apophysis (TA in Fig. 5) on the retrolateral side and both terminating below the upper portion containing tips of the terminal apophysis and the embolus; median apophysis (MA in Fig. 4) not as strongly developed compared to *Hogna lenta* (Hentz 1875), triangular in shape, projecting laterally toward the border of the cymbium and with a prominent ventrally directed spur (Figs. 4, 5). Epigynum of female shaped like an inverted T with an elongate median septum (MS in Fig. 19) or mid-field consisting of a longitudinal piece (LP in Fig. 18) and terminating in an elliptical or rounded trapezoidal transverse piece (TP in Fig. 18). Spermathecae (SP in Fig. 19) smooth and round to ovoid.



Figures 28, 29.—Dorsal view of *Tigrosa helluo*: 28. Female from Cold Spring Harbor, Suffolk County, New York. 29. Male from Saugerties, Ulster County, New York. Scale bar, 1 mm.



Figures 30–33.—*Tigrosa helluo*: Male from Saugerties, Ulster County, New York. 30. Left palpus, ventral view; 31. Left palpus, retrolateral view. 32, 33. Female from Cold Spring Harbor, Suffolk County, New York. 32. Vulva, dorsal view; 33. Epigynum, ventral view. Scale bars: palpi, 0.5 mm; epigyna, 0.1 mm.

KEY TO SPECIES

Females

1. Carapace with thin cream to yellow stripe beginning at AME row and ending at PLE row (Fig. 10). Transverse process (TP) of epigynum spade shaped; wide from anterior to posterior (Fig. 15). . . . . *Tigrosa aspersa*
   Carapace with thin, cream to yellow median longitudinal stripe beginning at AME row and continuing to posterior declivity (Figs. 3, 16, 22, 28). Transverse process (TP) of epigynum not spade shaped, more narrow from anterior to posterior (Figs. 9, 18, 26, 33) 2
2. Carapace with two short, cream to yellow dashes flanking the median stripe in the cephalic region (Fig. 3). Dorsum of abdomen with dark lanceolate cardiac mark enclosed by conspicuous broad cream to yellow stripes, and with paired cream to yellow dots or chevrons extending posteriorly to base of anal tubercle (Fig. 3). Venter of abdomen cream to pale yellow and without darker markings or with a few scattered spots. (Fig. 40) . . . . . *Tigrosa annexa*
   Carapace without conspicuous white dashes in cephalic area. Dorsum of abdomen more uniformly brown except for darker cardiac mark. Cardiac mark not surrounded by broad white stripes and with less conspicuous white dots or chevrons posteriorly. Venter of abdomen with darker spots or stripes (Figs. 41–43) 3
3. Occurring primarily west of the one hundredth meridian (Map 1). In ventral view the transverse piece (TP) of the epigynum, when measured from anterior to posterior, is greater than the narrow part of the longitudinal piece (LP) (Fig. 26). Internal genitalia (Fig. 27) distinct from *T. georgicola* (Fig. 19) and *T. helluo* (Fig. 32) . . . . . *Tigrosa grandis*
   Occurring primarily east of the one hundredth meridian (Maps 3, 5). In ventral view the TP of the epigynum, when measured from anterior to posterior, is about the same width as the narrow part of the LP (Figs. 18, 33). Internal genitalia (Figs. 19, 32) distinct from *T. grandis* (Fig. 27) 4
4. Large species (average body length over 20 mm). Carapace with broad, yellow to orange-brown, undulating submarginal stripes that often extend to lateral edges (Fig. 16). Venter of abdomen with rows of black to dark brown dots forming lines behind epigastric furrow and converging in front of spinnerets (Fig. 42). LP of epigynum flared outward anteriorly, exceeding half the width of the TP (Fig. 18) . . . . . *Tigrosa georgicola*
   Smaller species (average body length less than 15 mm). Carapace with thin pale yellow to orange submarginal stripes that are clearly separated from lateral edges (Fig 28). Venter of abdomen with scattered black dots (Fig. 41). LP of epigynum (Fig. 33) with sides,not flared outward anteriorly . . . . . *Tigrosa helluo*

Males

1. Carapace with cream to pale-yellow or orange submarginal stripes consisting of discontinuous irregular dashes (Fig. 11). Dorsum of abdomen with dark cardiac mark, not darkly outlined, but surrounded by a lighter background color with dark patches producing a mottled pattern (Fig. 11). Palpus with retrolateral piece of median apophysis not reaching edge of cymbium (Fig. 12) and palea rectangular in retrolateral view (Fig. 13) ..... *Tigrosa aspersa*
   Carapaee with eream to pale yellow orange submarginal stripes continuous from cephalic region to posterior edge (Figs. 2, 17, 23, 29). Dorsum of abdomen with dark cardiac mark outlined in dark brown to black (Figs. 1, 2, 17, 23, 29). Ventral view of palpus with retrolateral piece of median apophysis reaching edge of cymbium or beyond (Figs. 4, 6, 20, 24, 30). Palea not rectangular in retrolateral view of palpus (Figs. 5, 7, 21, 25) 2
2. Carapace with two conspicuous yellow longitudinal dashes in the cephalic region lateral to median stripe (Figs. 1, 2). Palea triangular in retrolateral view of palpus (Figs. 5, 7) ..... *Tigrosa annexa*
   Carapace without conspicuous yellow longitudinal dashes in the cephalic region lateral to median stripe (Figs. 10, 16, 22, 28). Palea with shape in retrolateral view as in Figs. 13, 21, 25, 31. 3
3. Occurring primarily west of the one hundredth meridian (Map 4). Carapace with yellow submarginal stripes beginning posterior to cephalic region and continuing to posterior edge (Fig. 23). Dorsum of abdomen with dark rectangular cardiac mark with posterior half outlined in black and surrounded by light tan color (Fig. 23). Venter of abdomen mottled in appearance without distinct dark brown to black dots or lines (Fig 44). Palea with shape in retrolateral view as in Fig. 25. ..... *Tigrosa graudis*
   Occurring primarily east of the one hundredth meridian (Maps 3, 5). Carapace with yellow submarginal stripes beginning in cephalic region and continuing to posterior edge (Figs. 17, 29). Dorsum of abdomen with dark lanceolate cardiac mark outlined in brown or black and surrounded by broad yellow stripes and with yellow chevrons or spots posterior to cardiac region (Figs. 17, 29). Venter with distinct dark brown or black spots (Fig. 41) or longitudinal lines of black spots (Fig. 42). Palea in retrolateral view of palpus as in Figs. 21 or 31 4
4. Large species (body length 25.4–33.6 mm). Broad cream to yellow submarginal stripes with uneven margins reaching edge of carapace (Fig. 17). Dorsum of abdomen with cardiac mark outlined in dark brown or blaek and enclosed by broad cream to yellow stripes (Fig. 17). Three or four dark chevrons posterior to cardiac region accented by light spots laterally. Venter cream to pale yellow with rows of black dots or dashes forming dark longitudinal lines (Fig. 42). Palea in retrolateral view of palpus as in Fig. 21 ..... *Tigrosa georgicola*
   Smaller species (Body length 9.6–12.9 mm) Thin cream to yellow submarginal stripes with edges not reaehing margins of earapace (Fig. 29). Dorsum of abdomen with cardiac mark darkly outlined, but with lighter surrounding area not as distinct as in *T. georgicola*) and darker chevrons posterior to cardiac region not as sharply defined (Fig. 29). Venter cream to pale yellow with scattered dark brown to black spots (Fig. 41). Palea in retrolateral view of palpus as in Fig. 31 ..... *Tigrosa helluo*

Tigrosa annexa (Chamberlin & Ivie 1944)
new combination
Figs. 1–9, 40, Map 1, Table 1

Lycosa helluo Gertsch 1934:6 (misidentified).
Lycosa annexa Chamberlin & Ivie 1944:142.
Hogna annexa Roewer 1955:257; Platnick 2011.

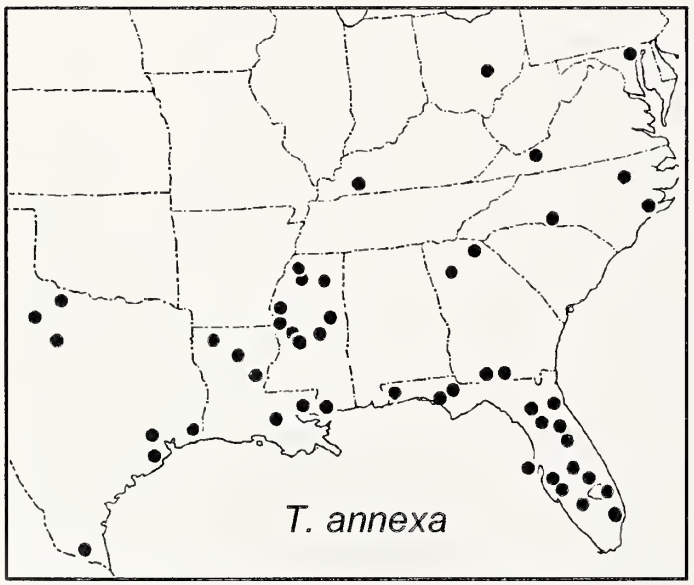
Type material.—Holotype: USA, Florida, Alachua County, Gainesville (29.65°N, 82.32°W), 10 February 1942, female, AMNH, examined.

Other material examined.—USA: *Ohio*, Perry Co., New Lexington (39.71N°, 82.21W°), no date, no name, AMNH, 1♀. *Maryland*, Hartford Co., Bel Air (39.54N°, 76.35W°), 1973, P. Morris, AMNH, 1♂. *Virginia*, Montgomery Co., Wildwood Park (37.14°N, 80.57°W), 4 May 1965, Hoffman, AMNH, 1♂. *Kentucky*, Christian Co., Hopkinsville (36.87°N, 87.49W°), no date, no name, AMNH, 1♂. *North Carolina*, Carteret Co., Duke Marine Laboratory, Piver Island (34.72°N, 76.66°W), 25 November 1981, R.D. Barnes, AMNH, 1♂, 4i; Edgecombe Co., 2 mi. [3.2 km] NW of Tarboro (35.91N°, 77.54°W), 13 June 1979, T.C. Lockley, HCC, 1♂, 2 mi. [3.2 km] NW of Tarboro, 14 June 1979, 2♂, 1i, 24 July 1979, 3♂, 25 July 1979, 1♂, 26 July 1979, 1♂, 8 September 1979, W.H. Cross, MSST, 1♂, 3 mi. [4.8 km] W of Tarboro (35.91°N, 77.54°W), 14 June 1979, 1i, 24 July 1979, 1♂, 1♀, 26 July 1979, 1♂, 8 September 1979, W.H. Cross, MSST, 1♂, 8 mi. [12.9 km] WSW of Tarboro (35.91°N, 77.54°W), 14 June 1979, 6♂, 25 July 5♂, 26 July 1979, W.H. Cross, MSST, 3♂; Union Co., Monroe (34.99°N, 80.55°W), 11 September 1942, Mrs. E.L. Bell, Jr., AMNH, 1♀. *Georgia*, Fulton and DeKalb Cos., Atlanta (33.75°N, 84.39W°), May 1989, no name, AMNH, 1♂; Lowndes Co., Valdosta (30.83N°, 83.28°W), 20–31 July 1916, F.E. Watson, AMNH, 1♀; Rabun Co., Lake Burton (34.86°N, 83.38°W), 29 June 1975, D.A. Rossman, AMNH, 1♂; Thomas Co., Bar M Ranch, 7 mi. [11.3 km] S of Boston (30.80°N, 83.79°W), 30 June 1973, A.R. Brady, HCC, 2♂, 2♀. Leaton Lake near Boston (30.79N, 83.79W), 27 July 1967, W. Sedgwick, MCZ, 1♀. *Florida*, Alachua Co., (29.68°N, 82.35°W), 25 Oetober 1932, H.K. Wallace, AMNH, 1♀, Gainesville (29.65°N, 82.32W°), 14 June 1935, 1♂, 1♀, 3i, W. Ivie & H. K. Wallace, AMNH, 4♂, 1♀, 10 February 1942, 1♂, 12 April 1943, 24 April 1943, 2♂, W. Ivie, AMNH, 2♂, 9 May 1958, H. V. Weems, Jr., AMNH, 1♂, Lake Lochloosa (29.50°N, 82.10°W), 7 April 1973, A. Jung, HCC, 2♂, 2♀; Bay Co., St. Andrews State Park (30.13°N, 85.74°W), 14 May 1987, 1♂, 11 May 1989, HCC, 1♂, 1♀; Hollywood (26.01°N, 80.16°W), 14 July 1935, H.K. Wallace, AMNH, 1♀; Dade Co., Miami (25.73N°, 80.24°W), 2 March 1936, S.C. Bishop, AMNH, 2♂, 1i, 5 April 1952, P. Porter, MCZ, 1♂; DeSoto Co., 8 mi. [12.9 km] W of Arcadia (27.22N°, 81.86°W), 31 March 1938, W.J. Gertsch, AMNH, 2♂, 6♀, 4i; Escambia Co., Pensacola (30.42°N, 87.22°W), 26 Novembcr 1944, D.C. Lowrie, AMNH, 2♀ with egg sacs; Hendry Co., 8 mi. [12.9 km] S of Moorehouse (26.57°N, 81.78°W), 25 April

Table 1.—Mean and range of ten females and ten males of *Tigrosa annexa* from Mississippi.

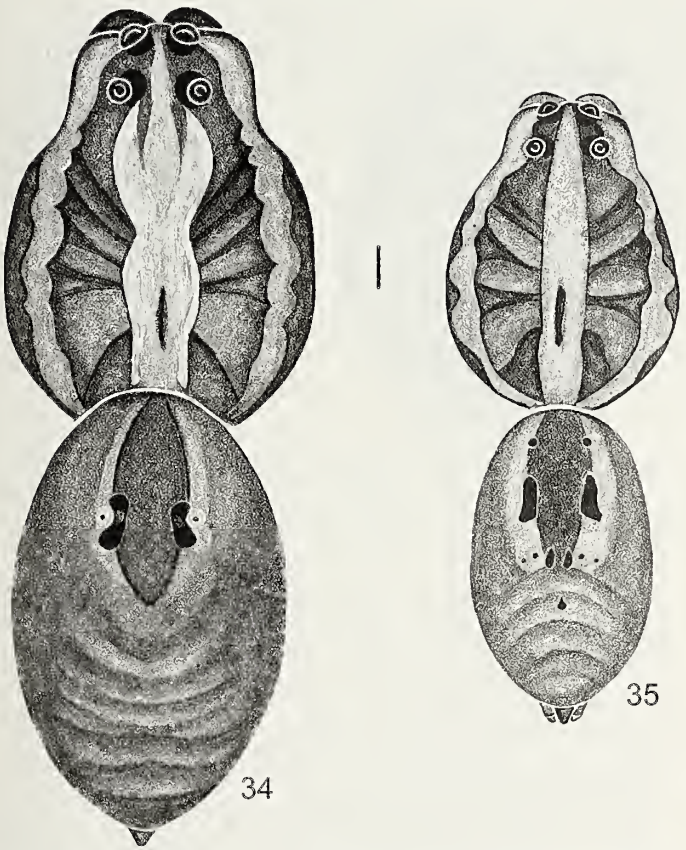
	Mean (range)		Mean (range)
Females			
Anterior eye row	1.19 (1.1–1.3)	Femur I	4.23 (3.9–4.9)
PME width	1.30 (1.2–1.4)	Patella-Tibia I	5.31 (4.7–6.3)
PLE width	1.65 (1.5–1.8)	Metatarsus I	2.77 (2.5–3.1)
POQ length	1.21 (1.1–1.4)	Tarsus I	2.00 (1.9–2.3)
Car. width at PLE	3.00 (2.8–3.3)	Total length I	14.30 (13.0–16.5)
Carapace width	4.64 (4.3–5.2)	Femur IV	4.81 (4.4–5.5)
Carapace length	6.09 (5.6–6.9)	Patella-Tibia IV	5.73 (5.3–6.5)
Body length	12.99 (10.8–15.6)	Metatarsus IV	4.97 (4.4–5.3)
Patella-Tibia II	4.69 (4.3–5.3)	Tarsus IV	2.46 (2.4–2.7)
Patella-Tibia III	4.11 (3.7–4.8)	Total length IV	17.96 (16.5–20.0)
Males			
Anterior eye row	1.24 (1.1–1.4)	Femur I	6.08 (5.1–7.7)
PME width	1.40 (1.2–1.7)	Patella-Tibia I	8.15 (6.4–10.4)
PLE width	1.83 (1.5–2.2)	Metatarsus I	5.51 (4.3–6.9)
POQ length	1.28 (1.1–1.5)	Tarsus I	3.37 (2.7–4.1)
Car. width at PLE	2.95 (2.4–3.6)	Total length I	23.10 (18.4–29.0)
Carapace width	5.37 (4.4–6.7)	Femur IV	6.69 (5.3–8.1)
Carapace length	6.96 (5.6–6.7)	Patella-Tibia IV	8.19 (6.7–10.2)
Body length	13.38 (10.5–17.4)	Metatarsus IV	7.90 (6.3–10.0)
Patella-Tibia II	7.04 (5.5–8.9)	Tarsus IV	3.39 (2.8–4.1)
Patella-Tibia III	6.09 (4.9–7.7)	Total Length IV	26.17 (21.3–32.1)

1952, A. Schwartz, MCZ, 1♂; Lee Co., Fort Myers (26.64°N, 81.87°W), 18 March 1954, W. Ivie, AMNH, 1♂, 1♀, 1i; Liberty Co., Torreya State Park (30.57°N, 85.74°W), 23 June 1997, A.R. Brady, K.A. Brewer, A.C. Wyatt, HCC, 1♀; Martin Co., Port Mayaca (26.99°N, 80.61W°), 29 March 1938, W.J. Gertsch, AMNH, 2♂, 5♀, 3i; Okeechobee Co., Okeechobee (27.98°N, 81.54°W), 28 March 1938, 13♂, 19♀, 18i, 29 March, W.J. Gertsch, AMNH, 2♂, 13♀, 1i; Orange Co., Winter Park (28.59°N, 81.35W°), 21 March 1938, W.J. Gertsch, AMNH, 4♀, 2i; Pinellas Co., St. Petersburg (27.77N°, 82.64W°), 30 November 1933, H.K. Wallace, AMNH, 2♂, 2♀; Polk Co., Poe Springs , Santa Fe River (27.66°N, 81.52°W), 17 March 1934, H.K. Wallace, AMNH, 2♂; Putnam Co, Welaka Reserve

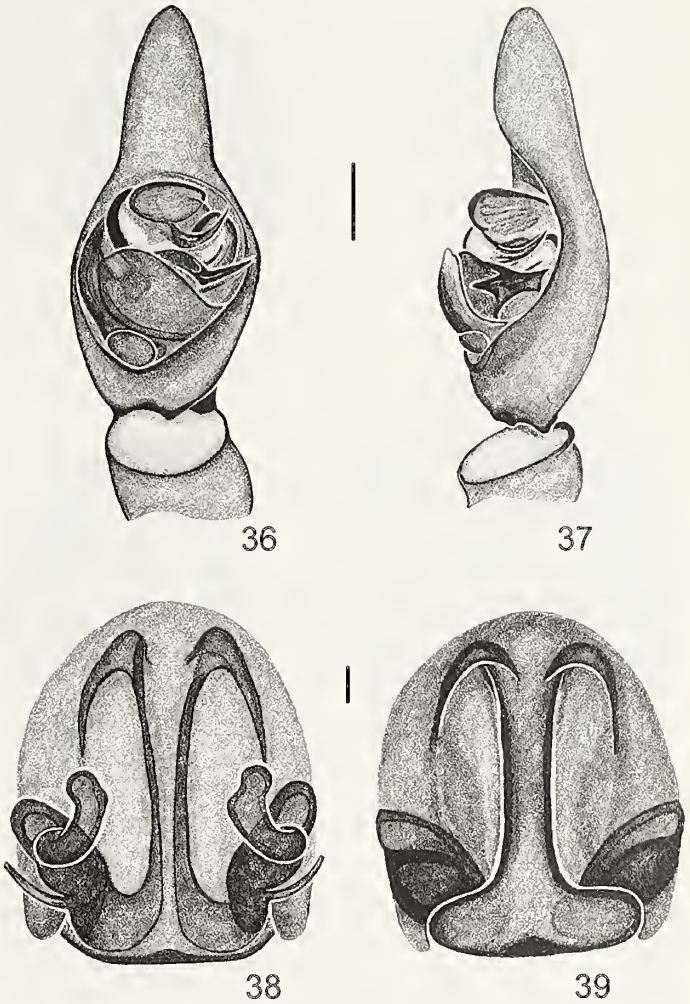


Map 1.—Distribution Map of *Tigrosa annexa*.

(29.48°N, 81.67°W), 18 May 1980, 1♂, 2i, 22 May 1987, 1♀, 20 May 1988, 1♀, 19 May 1989, A.R. Brady, HCC, 1♀; Seminole Co., Sanford (28.80°N, 81.28°W), W.H. & L.F. Stickel, AMNH, 5♂, 3i; Volusia Co., Deland (29.03°N, 81.31W°), 25 March 1939, F.E. Lutz, AMNH, 1♀. *Mississippi,* Hancock Co., Bayou La Croix, 3 mi. [4.8 km] N of Waveland (30.31N°, 89.37°W), 12 June 1982, M.W. LaSalle, MSST, 1♂, 4♀; Madison Co., (32.64N°, 90.09°W), 7–8 mi. [11.3–12.9 km] W of Interstate Highway 55 on Gluckstadt Road, 9–12 September 1982, T.C. Lockley, HCC, 1♂; Newton Co., 1 mi. [1.6 km] E of Union (32.57°N, 89.12°W), 29 August 1982, 1♀, 2–4 July 1983, 7♂, 2♀, 3–5 September 1980, T.C. Lockley, HCC, 5♀; Oktibbeha Co., Craig Springs (33.32°N, 88.92°W), 3 October 1979, G.L. Snodgrass, MSST, 1♂, Mississippi State University, Starkville (33.46°N, 88.79°W), 28 July 1983, B. Booth, MSST, 1♂; Panola Co., 11 mi. [17.7 km] WSW of Batesville (34.32°N, 89.95°W), 21 June 1979, W.H. Cross, MSST, 2♂, 1♀, 6 mi. [9.7 km] SW of Como (34.51°N, 89.94°W), 21 June 1979, W.H. Cross, MSST, 2♂, 3 mi. [4.8 km] WSW of Sardis (34.44°N, 89.92°W), 21 June 1979, 1♂, 2 August 1979, W.H. Cross, MSST, 1♂; *Pontotoc Co.*: 1 mi. [1.6 km] SE of Ecru (34.35N°, 89.03°W), 24 April 1980, 1♂, 5 June 1980, 2♂, 1♀, 19 June 1980, 1♀, 3 July 1980, 2♂, 16 July 1980, 1♀, 12 September 1980, 24 October 1980, W.H. Cross, MSST, 1♂; Washington Co. (33.30°N, 90.94°W), 13–14 July 1982, T.C. Lockley, HCC, 2♂; Leroy Percy State Park (33.16°N, 90.94°W), May 1983, T.C. Lockley, HCC, 1♂; 1 mi. [1.6 km] N of Stoneville (33.42°N, 90.92°W), 31 January–2 February 1983, T.C. Lockley, HCC, 1♂, 1♀, 2 mi. [3.2 km] N of Stoneville (33.42°N, 40.92°W), 22 June 1982, 1♂, 13–14 July 1982, 2♂, 17–20 September 1982, 1♂, 24–27 September 1982, 22–24, December 1982, 1i, HCC, 1♂, 3–6 June 1983, 1–6 July 1983, 1♂, T.C. Lockley, HCC, 1♂; Yazoo Co. (32.76°N, 90.36°W), 23 October 1964, 1♀, 20 March 1♀, 15 July 1♂, P.R. Dorris, MSST, 1♂. *Louisiana,* Catahoula Par., Camp Plauche (31.24°N, 92.15°W), 12 February 1944, D.E. Beck, AMNH, 1♂; East



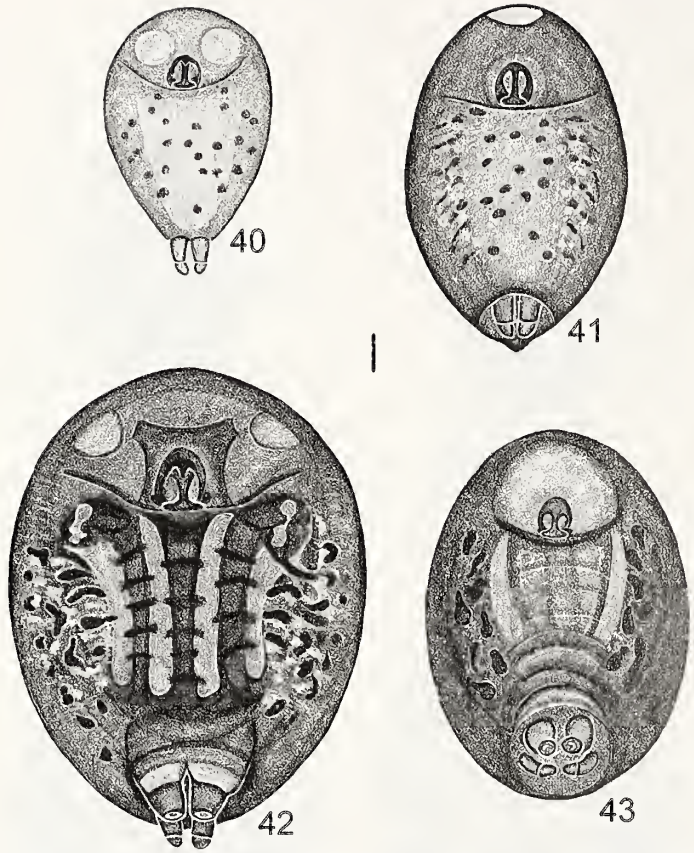
Figures 34, 35.—Dorsal view of *Hogna radiata*: 34. Female from Cerbére, Peyrefite Bay, France; 35. Male from Cerbére, Peyrefite Bay, France. Scale bar, 1 mm.



Figures 36–39.—*Hogna radiata*: 36, 37. Male from Cerbére, Peyrefite Bay, France. 36. Left palpus, ventral view; 37. Left palpus, retrolateral view. 38, 39. Female from Cerbére, Peyrefite Bay, France. 38. Vulva, dorsal view; 39. Epigynum, ventral view. Scale bars: palpi, 0.5 mm; epigyna, 0.1 mm.

Baton Rogue Par., Baton Rogue (30.97°N, 91.52°W), August 1956, no name, AMNH, 2♀, 10 August 1975, F.W. Howard, AMNH, 1♂; Iberville Par., St. Gabriel (30.26°N, 91.10°W), 1 July 1972, 1♂, 1♀, 1i, 13 July 1972 3♂, 2♀ 5i, 22 July 1972, 1♀, 23 July 1972, 1♀, 2i, 1 August 1972, 6♀, 8i, 9 August 1972, 1♂, 9♀, 21 September 1972, 3♀, 6i, 23 July 1972, 2♀, 2i26 September 1972, 2♀, 4 October 1972, 1♂, 2♀, 6i, 21 November 1972, 1♀, 4 December 1972, 1♂, 2♀, 3i, 19 June 1973, 3♂, 2♀, 5i, 17 August 1973, 1♂, 6♀, 4i , 24 August 1973, 1♂, 3♀, 3i, 1 September 1973, F.W. Howard, AMNH, 2♂, 6♀, 1i; Madison Par., Tallulah (32.41N°, 91.19°W), 15 July 1945, no name, AMNH, 1♀; Ouachita Par., Monroe (32.51N°, 92.12°W), no name, AMNH, 1♂; St. Tammany Par., Herbert Natural Preserve (30.97°N, 91.52°W), 23 June 1984, A.R. Brady, HCC, 1♂. *Texas*, Brazoria Co., Brookside Village (27.59°N, 95.31°W), 27 December 1984, no name, MWSU, 1♀; Harris Co., Houston (29.76°N, 95.38°W), June 1954, E. Stude, AMNH, 1♂, Haskell Co., 10 mi. [16.1 km] W of Rochester (33.32°N, 99.85°W), no date, F.D. White, MWSU, 1♀, 12 mi. [19.3 km] W of Rochester (33.32°N, 99.85°W), 17 April 1977, F.D. White, MWSU, 2♀, Hidalgo Co., (26.41°N, 98.22°W), 2 July 1934, S. Mulaik, AMNH, 1♂, 3♀, Edinburg (26.30°N, 98.16°W), 1–10 December 1936, S. Mulaik, AMNH, 1♂, 1♀; Harris Co., Houston (29.36°N, 95.37°W), July 1939, J.H.S., AMNH, 1♀, Edinburg (26.30°N, 98.16°W), 4 December 1935, M. Welch, AMNH, 1♀, 5 December 1936, S. Mulaik, AMNH, 1♂; Jefferson Co., Port Arthur (29.88°N, 93.94°W), 1 May 1944, 2i, 11–18 May 1944, E.D. Palmer, AMNH 1♀; Palo Pinto Co., Palo Pinto (32.77°N, 98.30°W), March 1973, T. Salmon, MWSU, 1♀; Wichita Co., (34.03°N, 98.80°W), 20 July 1975, J. Cokendolpher, MWSU, 1♀, 13 September 1977, N.V. Horner, MWSU, 1♀, 20 February 1981, G.J. Merchant, MWSU, 1i, Lake Wichita (33.84°N, 98.53°W) 7 April 1980, G. J. Merchant, MWSU, 1♀, 20 February 1981, G.J. Merchant, MWSU, 1♀.

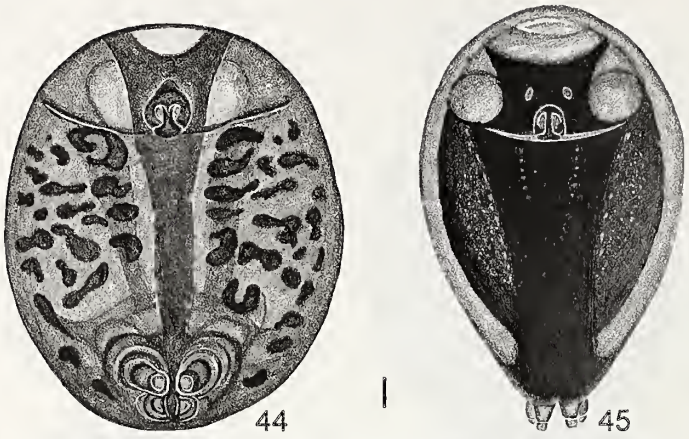
Diagnosis.—*Tigrosa annexa* is most closely related to *T. helluo* in size as well as male and female genitalic characters. The dorsal color pattern on the carapace and abdomen of *T. annexa* readily distinguishes this species from *T. helluo* and *T. georgicola.* There are two white dashes just behind the PME in *T. annexa* (Figs. 1–3) not seen in *T. helluo* (Figs. 28, 29) or *T. georgicola* (Figs. 16, 17). Also in *T. annexa* there are broad white stripes surrounding the dark cardiac mark and a paired series of four large white spots beginning in the cardiac area and extending posteriorly to the base of the spinnerets not seen in *T. helluo* or *T. georgicola.* In addition *T. annexa* is the only species of *Tigrosa* in which the venter of the abdomen is lighter cream colored with only a few insignificant darker spots and no stripes or other markings (Fig. 40).



Figures 40–43.—Ventral view of female abdomens: 40. *Tigrosa annexa* from Bar M Ranch, 7 mi. [11.3 km] S of Boston, Thomas County, Georgia. 41. *Tigrosa helluo* from Horseshoe Bend of Neshaminy Creek, E of Jamison, Bucks County, Pennsylvania; 42. *Tigrosa georgicola* from Torreya State Park, 15 mi. [24.1 km] N of Bristol, Liberty County, Florida; 43. *Tigrosa grandis* from Ft. Collins, Larimer County, Colorado. Scale bar, 1 mm.

Remarks.—Before Chamberlin & Ivie (1944) first described *T. annexa*, this species was often confused with *T. helluo* and *T. georgicola*. Females of *T. georgicola* (body length 16.6–22.2 mm) are much larger than females of *T. annexa* (body length 10.8–15.6 mm) that I have examined. However, among collections of *T. annexa* from Mississippi I have found very large males, which overlap in size with males of *T. georgicola*. It is possible that these large males of *T. annexa* represent a different species, but they are indistinguishable from smaller specimens except in size and I have not identified a comparable larger female.

Color pattern.—*Female:* Dorsal pattern illustrated in Fig. 3. Face with lower part orange-yellow to yellow and upper part dark brown to black. Eye region dark brown to black with a thin yellow line from AME to PLE. Chelicerae orange-brown to dark reddish brown. Carapace brown with narrow median yellow stripe. Two short lighter yellow dashes originating behind PLE and extending to posterior cephalic region. Narrow yellow submarginal stripes with scalloped or uneven margins and more narrow than median stripe in thoracic region. Dorsum of abdomen dark brown with dark lanceolate cardiac mark outlined in black and bordered by lighter yellow color. Lateral areas of abdomen darker brown with pairs of conspicuous paired yellow spots extending from



Figures 44, 45.—Ventral view of abdomen: 44. *Tigrosa aspersa* from Imboden, Lawrence County, Arkansas; 45. *Hogna radiata* from Island of Sardinia, Italy. Scale bar, 1 mm.

cardiac area to base of anal tubercle. Venter cream to pale yellow without darker markings or with a few scattered dark spots. Legs yellow on dorsal surface with pale yellow to cream ventrally, without darker bands or markings. Labium and endites pale yellow to brown with distal ends lighter yellow to cream. Sternum yellow to brown, often with a lighter median dash.

Male. Dorsal pattern illustrated in Figs. 1, 2. Face orange-yellow to yellow, darker brown in eye region with nacelles circled in black and line of white hair between PME. Condyles yellow-orange to darker brown. Chelicerae yellow orange to orange brown. Carapace with eyes circled in black, background color brown with black lines radiating from fovea. Narrow median yellow stripe from PLE to posterior declivity. Cephalic region with short yellow dashes from PLE converging on median stripe. Submarginal yellow stripes with uneven edges. Abdomen with dark brown lanceolate cardiac mark outlined in black and accented in yellow. Lateral regions of abdomen medium to dark brown. Median area posterior to cardiac region yellow and traversed by four dark brown chevrons. Venter of abdomen pale yellow to cream with a few scattered small dark spots. Legs yellow to yellow-orange without darker markings; ventral surfaces a shade lighter. Labium and endites cream to pale yellow with distal ends lighter. Sternum cream to yellow with a pair of faint dusky dashes.

Natural history.—*Tigrosa annexa* was described by Gertsch (1934) as a light variety of *T. helluo*. Because *T. annexa* was often confused with *T. helluo* and sometimes even with *T. georgicola*, a much larger species, it was overlooked in collections, and the differences in habitat and behavior of this species were not noted. In collections that I have examined from Mississippi many of the *T. annexa* specimens were taken from pitfall traps in cotton fields or peripheral to cotton fields, and a smaller number from herbage, such as Bermuda grass. Numerous specimens of *T. annexa* collected by F.W. Howard from St. Gabriel, Louisiana, and housed in the AMNH were taken from pitfall traps in Bermuda grass. *Tigrosa helluo* is often found in wetter habitats than *T. annexa*, such as bogs in Michigan or in plant growth near lakes or swampy areas in the southeastern United States.

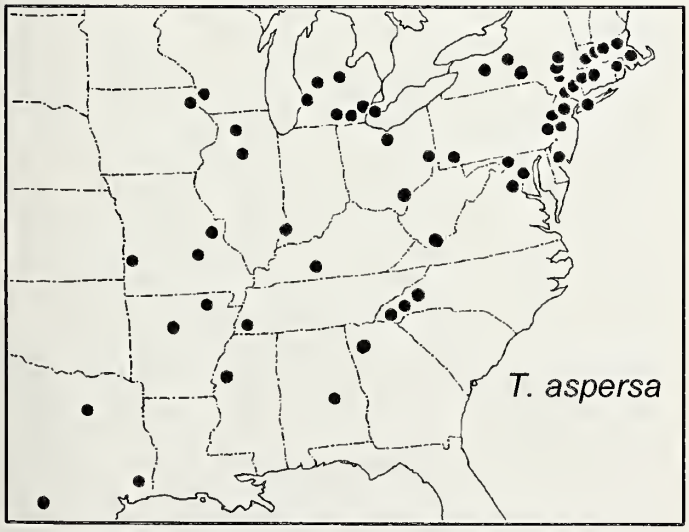
Table 2.—Mean and range of ten females and ten males of *Tigrosa aspersa* from Arkansas.

	Mean (range)		Mean (range)
Females			
Anterior eye row	2.06 (1.9–2.2)	Femur I	8.98 (8.4–9.6)
PME width	2.11 (2.0–2.3)	Patella-Tibia I	11.32 (10.6–12.0)
PLE width	2.95 (2.8–3.2)	Metatarsus I	6.65 (6.0–7.3)
POQ length	1.93 (1.8–2.0)	Tarsus I	3.90 (3.7–4.0)
Car. width at PLE	6.21 (5.6–6.7)	Total length I	30.84 (28.7–32.9)
Carapace width	9.34 (8.5–10.1)	Femur IV	9.88 (9.3–10.5)
Carapace length	12.57 (12.0–13.3)	Patella-Tibia IV	11.37 (10.6–12.1)
Body length	27.86 (25.0–30.5)	Metatarsus IV	10.16 (9.2–11.3)
Patella-Tibia II	10.09 (9.4–11.6)	Tarsus IV	4.16 (3.7–4.7)
Patella-Tibia III	8.66 (7.8–9.3)	Total length IV	35.58 (33.1–38.4)
Males			
Anterior eye row	1.74 (1.7–1.9)	Femur I	8.91 (8.0–9.3)
PME width	1.87 (1.7–2.0)	Patella-Tibia I	11.74 (11.2–12.5)
PLE width	2.47 (2.3–2.6)	Metatarsus I	8.13 (6.9–9.2)
POQ length	1.72 (1.5–1.9)	Tarsus I	4.69 (4.3–5.2)
Car. width at PLE	4.63 (4.1–5.1)	Total length I	33.53 (29.3–36.2)
Carapace width	8.05 (7.2–8.9)	Femur IV	9.70 (8.4–10.6)
Carapace length	10.25 (9.3–11.7)	Patella-Tibia IV	11.70 (10.8–13.0)
Body length	19.87 (18.5–21.3)	Metatarsus IV	11.70 (10.6–12.9)
Patella-Tibia II	10.47 (9.3–11.0)	Tarsus IV	4.99 (4.3–5.7)
Patella-Tibia III	8.96 (8.1–9.8)	Total Length IV	38.12 (34.0–42.3)

Distribution.—*Tigrosa annexa* has been found along the Atlantic coast from Delaware south to Big Pine Key, Florida, and westward from southern Ohio to the southern tip of Texas (Map 1).

Tigrosa aspersa (Hentz 1844)
new combination
Figs. 10–15, 44, Map 2, Table 2

Lycosa aspersa Hentz 1844:389; Chamberlin 1908:236; Kaston 1948:323.
Tarentula inhonesta Keyserling 1877:634.
Tarentula tigrina McCook 1879:xi; Stone 1890:423.
Lycosa vulpina Emerton 1885:487.
Lycosa immaculata Banks 1892:67.
Lycosa exitiosa Banks 1892:68.
Lycosa oblonga Banks 1892:68.



Map 2.—Distribution Map of *Tigrosa aspersa*.

Lycosa inhonesta Montgomery 1902:557; 1904:290.
Hygrolycosa aspersa Roewer 1955:261.
Hogna aspersa Dondale & Redner 1990:49; Platnick 2011.

Type material.—Holotype: USA: Alabama: Specimen lost.
Other material examined: CANADA: *Ontario*, Pelee Island (41.77°N, 82.69°W), 4–16 June 1950, W. Ivie & T. E. Kurata, AMNH, 1♀, Windsor (42.32°N, 83.03°W), 1 September 1953, R. Barrett, AMNH, 1♀. USA: *Massachusetts*, Franklin Co., Mount Toby near Sunderland (42.47°N, 72.58°W), H., L. & F. Levi, MCZ, 1♀; Middlesex Co., Cambridge, (42.37°N, 71.11°W), August 1916, C. Mason, MCZ, 1♂, Pepperell (42.67°N, 71.59°W), August 1964, H.W. Levi, MCZ, 1♀, September 1968, H. & L. Levi, MCZ, 1♀, August 1971, H., L. & F. Levi, August 1971, MCZ, 1♀, Townsend (42.67°N, 71.70°W), no date, H.W. Levi, MCZ, 1♀, Woburn (42.48°N, 71.15°W), no date, J.G. Shute, MCZ, 1♀; Plymouth Co., Marshfield (42.09°N, 70.71°W), 18 June 1933, no name, MCZ, 1♂. *Rhode Island*, Providence Co., Providence (41.82°N, 71.41°W), no date, N. Banks, MCZ, 1♀. *Connecticut*, Fairfield Co., New Canaan (41.15°N, 73.49°W), 1–15 September 1950, no name, MCZ, 2♂, 30 September 1950, no name, MCZ, 1♀; Hartford Co., Unionville (41.76°N, 72.89°W), 8 September 1967, D. Goellner, MCZ, 1♀; Litchfield Co., Goshen (41.83°N, 73.23°W), no date, J. H. Emerton, MCZ, 1♂, New Haven Co., Bethany (41.42°N, 73.00°W), 29 May 1933, B.J. Kaston, MCZ, 2♀, 15 August 1939, D.S. Riggs, MCZ, 1♀. *New York*: Albany Co., Rensselaerville (42.52°N, 74.14°W), 4 July 1967, R. & J. Matthews, MCZ, 1♀; Greene Co., Leeds (42.52°N, 74.14°W), September 1931, D.B. Merriam, AMNH, 2♀; Onondaga Co., Syracuse (43.05°N, 76.15°W), no date, J.H. Emerton, MCZ, 1♀; Ontario Co., Canandaigua Lake near Woodville (42.89°N, 77.28°W), 16 September 1939, S.C. Bishop, AMNH, 1♂; Rockland Co., Sloatsburg (41.16°N,

74.19°W), 20 September 1934, W.J. Gertsch, 1♀; Suffolk Co., Port Jefferson (40.95°N, 73.07°W), September 1954, W.J. Gertsch, AMNH, 1♂; Tompkins Co., Ithaca (42.44°N, 74.19°W), September 1954, W.J. Gertsch, AMNH, 1♀, 4 September 1976, G. Dingerkus, AMNH, 2♀, 19 September 1976, G. Dingerkus, W. Dun & D. Denihy, AMNH, 1♀; Ulster Co., Lake Minnewasha (40.74°N, 74.24°W), no date, H.P. Curtis, MCZ, 1 ♀, Lake Minnewasha , Loft Mountain, Camp Shenandoah (41.53°N, 73.79°W), 18 October 1966, F. Beer, AMNH, 1♀; Westchester Co., Hartsdale (41.01°N, 73.80°W), 27 August 1945, W.H. Ingram, AMNH, 1♀, Montrose Point (42.25°N, 73.93°W), 10 September 1948, M. Thurston, AMNH, 1♂, South Berne (42.56°N, 74.10°W), no date, no collector, AMNH, 1♀. *New Jersey*, Bergen Co., Ramsay (40.14°N, 74.73°W), 3 September 1936, W.J. Gertsch, AMNH, 1♂, 18 September 1946,W.J. Gertsch, AMNH, 2♀; Cumberland Co., Vineland (39.49°N, 75.03°W), no date, Treat, MCZ, 1♂; Somerset Co., Neshanic (40.50°N, 74.72°W), no date, E.S. Gaffney, AMNH, 2♀; Warren Co., Washington (40.76°N, 74.98°W), 20 September 1943, E.A. Rose, AMNH, 1♀. *Pennsylvania*, Bucks Co., NE of Jamison (40.16°N, 75.03°W), October 1963, M. Hunting, AMNH, 1♀; Fayette Co., Dunbar (39.98°N, 79.62°W), 30 June 1932, O. Greenwood, AMNH, 1♀. *Maryland,* Frederick Co., Myersville (39.51°N, 77.57°W), 2 September 1915, Hyslop & Parker, MCZ, 1♀; Montgomery Co., Kensington (39.03°N, 77.07°W), 28 May 1944, J.M. Paris, AMNH, 1♀. *Ohio*, Athens Co., Athens (39.33°N, 82.10°W), 24 September 1938, W.C. Stokes, AMNH, ♀. *West Virginia,* Mercer Co., Princeton (37.37°N, 81.10°W), 14 March 1969, N.I. Platnick, AMNH, 1♀; Ohio Co., Wheeling (40.06°N, 80.72°W), August–October 1947, K.W. Haller, 1♀, 20 October 1954, K.W. Haller, AMNH, 1♀. *Virginia,* Falls Church (Independent City) (38.89°N, W80.59°W), no date, E.B. Bryant, MCZ, 1♂. *Kentucky*, Edmunson Co., Bee Springs (37.29°N, 86.28°W), 8 June 1974, Sanborn, MCZ, 1♀. *Tennessee,* De Kalb Co., Shelby Forest State Park (35.83°N, 85.98°W), 9 September 1958, R. Wiley, MCZ, 1♀. *North Carolina,* Buncombe Co., Black Mountain (35.62°N, 82.32°W), no date, N. Banks, MCZ, 1♀; Cherokee Co., Murphy (35.09°N, 84.03°W), 23 July 1903, N. Banks, MCZ, 1♀; Jackson Co., Blue Ridge Parkway at Rattlesnake Mountain (35.62°N, 83.28°W), J. & W. Ivie, 15 October 1965, AMNH, 1♂. *Georgia*, DeKalb County, Roosevelt State Park near Pine Mountain (32.86°N, 84.72°W), no name, 1 August 1960, AMNH, 1♀ with egg sac. *Mississippi,* Washington Co., Greenville (33.41°N, 91.06°W), 15 August 1985, P. Wilcox, AMNH, 1♂. *Alabama,* Montgomery Co., Montgomery (32.37°N, 86.30°W), September 1948, C.V. Lopp, AMNH, 2♂. *Louisiana,* Orleans Par. (30.97°N, 91.52°W), 16 January 1920, H.E. Hubert, AMNH, 1 ♀ with egg sac. *Michigan*, Calhoun Co., Albion (42.25°N, 84.75°W), 2 October 1933, 1♀, September 1935, A.M. Chickering, MCZ, 1♂; Livingston Co., E.S. George Reserve (42.46°N, 83.95°W), 29 May 1955, I.J. Cantrall, FSCA, 1♀; Midland Co., (43.65°N, 84.39°W), 4 August 1942, A.M. Chickering, MCZ, 1♀; Newaygo Co., Manistee National Forest (43.50°N, 82.57°W), 3 October 1974, S. Scholl, HCC, 1♂; Oceana Co., 5 mi. [8.0 km] SE of Whitehall (43.41°N, 86.34°W), July 1938, M. Heifetz, AMNH, 1♀; Ottawa Co., Holland (42.79°N, 86.11°W), September 1986, A.R. Brady, HCC, 1♀; Ivan Buren Co., Van Buren State Park (42.33°N, 86.30°W), 1 October 1930, F.J. Hermann, AMNH, 1♀; Washtenaw Co., Ann Arbor (42.28°N, 83.75°W), 29 September 1932, A.M. Chickering, MCZ, 1♀, Chelsea (42.32°N, 84.02°W), 5 September 1978, J. Hodge, AMNH, 1♂; Wayne Co., Ecore (43.74°N, 83.15°W), September 1933, H.M. Zeerman, MCZ, 1♀. *Indiana*, La Porte Co., Smith (40.44°N, 79.24°W), 27 July 1935, D.C. Lowrie, AMNH, 1♀; Posey Co., New Harmony (38.13°N, 87.93°W), no date, N. Banks, MCZ, 2♂. *Wisconsin*: Crawford Co., Gays Mills (43.32°N, 90.84°W), August 1950, L. Kegel, MCZ, 1♀. *Illinois,* La Salle Co., Tonica (41.22°N, 89.07°W), 4 August 1932, W.J. Gertsch, AMNH, 1♀; Ogle Co. (42.05°N, 89.31°W), June, J.A. Allen, MCZ, 2♀. *Iowa*, Clayton Co., McGregor (43.02°N, 91.18°W), no date, no name, MCZ, 1♀. *Missouri,* Crawford Co., 5 mi. [8.0 km] W. of Berryman (37.92°N, 91.10°W), 2 April 1955, R. Crabill, AMNH, 1♀; St. Louis Co., St. Louis (38.63°N, 90.20°W), September 1961, J. Gerard, AMNH, 1♀; Vernon Co., Nevada (37.84°N, 94.35°W), 16 September, 1961, D. & J. McReynolds, MCZ, 1♂, 17 September 1976, D. Lamore , MCZ, 1♀. *Arkansas*, Lawrence Co., Imboden (36.20°N, 91.17°W), 1935, B.C. Marshall, AMNH, 5♂, 7♀; Van Buren Co., Little Red River (34.75°N, 92.13°W), 18 July 1961, no name, MCZ, 1♀. *Texas*, Bexar Co., San Antonio (29.42°N, 98.49°W), 1936, A. Vick, AMNH, 1♀; Dallas Co., (32.80°N, 96.84°W), 5 June 1944, S.E. Jones, MCZ, 1♀.

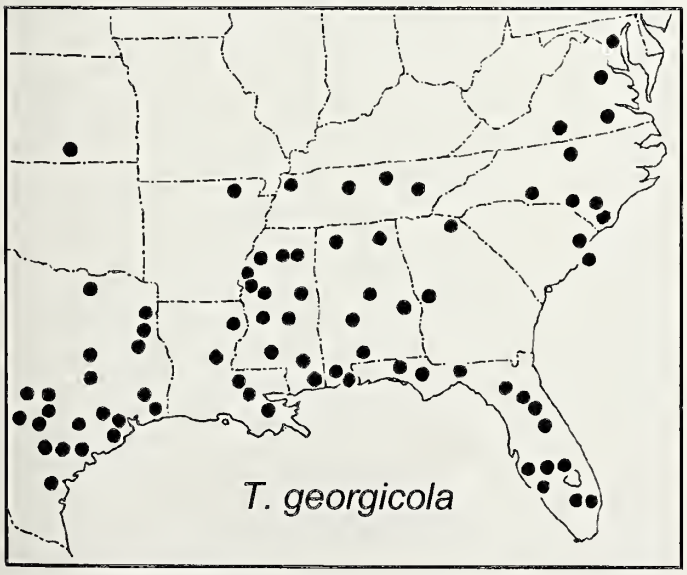
Diagnosis.—*Tigrosa aspersa* can be distinguished from *T. helluo* and *T. annexa* by its larger size. Comparisons of the body lengths of females illustrate these size differences. The mean sizes for the three species are *T. aspersa*, 28 mm; *T. helluo*, 21 mm; *T. annexa*, 13 mm. Size differences can also be seen in carapace widths, dimensions of the eye rows, and leg lengths (Tables 1–3). In *T. aspersa* the restriction of the pale median stripe to the eye region of the cephalothorax in the female (Fig. 10) is a character that distinguishes it from *T. georgicola* (Fig. 16) and *T. grandis* (Fig. 22). In addition the lighter submarginal stripes on the carapace in *T. aspersa* females (Fig. 10) are often broken into shorter segments and appear much less distinct than in females of other species. The LP of the epigynum in *T. aspersa* (Fig. 15) is about as long as the width of the TP, while in *T. georgicola* (Fig. 18) and *T. grandis* (Fig. 26) the LP is longer than the width of the TP. Also the epigynum of *T. aspersa* is spade-shaped and stouter from anterior to posterior than in *T. georgicola* or in *T. grandis*. Male *T. aspersa* have the submarginal stripes on the carapace separated into shorter segments (Fig. 11), unlike *T. georgicola* (Fig. 17) and *T. grandis* (Fig. 23), where they are continuous. The dorsal pattern on the abdomen of *T. grandis* is mottled without distinct chevrons (Fig. 11), while in *T. georgicola* there are broad lighter stripes surrounding the dark cardiac area followed by four to five distinct darker chevrons posterior to the cardiac area (Fig. 17), and in *T. grandis* four to five darker chevrons are usually visible, but lighter markings surrounding the cardiac area are absent. The median apophysis of *T. aspersa* (Fig. 12) is shorter and less developed

Table 3.—Mean and range of ten females and ten males of *Tigrosa georgicola* from Mississippi.

	Mean (range)		Mean (range)
Females			
Anterior eye row	1.63 (1.4–1.9)	Femur I	7.02 (5.9–8.2)
PME width	1.80 (1.6–2.0)	Patella-Tibia I	9.06 (7.4–10.6)
PLE width	2.34 (1.9–2.7)	Metatarsus I	5.36 (4.3–6.1)
POQ length	1.64 (1.4–1.9)	Tarsus I	3.34 (2.7–4.0)
Car. width at PLE	4.43 (3.6–5.7)	Total length I	24.88 (20.2–30.1)
Carapace width	7.13 (5.7–8.6)	Femur IV	7.69 (6.3–9.3)
Carapace length	9.58 (7.6–11.4)	Patella-Tibia IV	9.46 (7.8–10.9)
Body length	20.54 (16.6–22.2)	Metatarsus IV	8.74 (7.7–9.6)
Patella-Tibia II	8.14 (6.7–9.6)	Tarsus IV	3.78 (3.3–4.1)
Patella-Tibia III	6.97 (5.6–8.2)	Total length IV	29.66 (25.1–33.8)
Males			
Anterior eye row	1.32 (1.2–1.6)	Femur I	6.70 (5.3–8.0)
PME width	1.49 (1.3–1.8)	Patella-Tibia I	8.78 (7.2–10.2)
PLE width	1.93 (1.6–2.2)	Metatarsus I	6.26 (5.3–7.2)
POQ length	1.36 (1.2–1.7)	Tarsus I	3.95 (3.3–4.7)
Car. width at PLE	3.21 (2.5–3.9)	Total length I	25.70 (21.1–29.8)
Carapace width	5.64 (4.3–6.8)	Femur IV	7.46 (6.4–8.8)
Carapace length	7.43 (5.6–9.3)	Patella-Tibia IV	8.80 (7.6–10.0)
Body length	14.20 (10.6–17.2)	Metatarsus IV	8.96 (8.0–10.2)
Patella-Tibia II	7.78 (6.4–9.2)	Tarsus IV	3.98 (3.5–4.8)
Patella-Tibia III	6.66 (5.3–8.0)	Total Length IV	29.21 (25.4–33.6)

than in *T. georgicola* (Fig. 20). In *T. aspersa* the palea (Fig. 13) is comparatively smaller than in *T. georgicola* (Fig. 21), and the sclerotized ridges are less prominent in *T. aspersa.*

Color pattern.—*Female:* Dorsal pattern illustrated in Fig. 10. Face reddish brown, ALE row with black nacelles. Chelicerae dark brown to black. Carapace dark reddish brown (mahogany), with black lines radiating from black thoracic groove. Faint lighter brown color forming broken or interrupted submarginal stripes. Dorsum of abdomen brown with only slightly darker stripes surrounding cardiac region. Sometimes with two light spots along anterior region of cardiac area. Four to five slightly darker chevrons on posterior third of abdomen. Venter of abdomen with median longitudinal region light brown. Lateral areas mottled, yellow background covered with dark brown irregular spots (Fig. 44). Legs with prominent dark bands; usually two prominent dark bands on femora, tibiae and metatarsi alternating with two narrow lighter yellowish bands. Thick white scopulae on ventral surfaces of tarsus and metatarsus; dense on legs I and II. Labium and endites dark brown with yellow distal ends. Sternum dark brown without lighter markings. Spinnerets brownish yellow.



Map 3.—Distribution Map of *Tigrosa georgicola.*

Male: Dorsal pattern illustrated in Fig. 11. Face orange to reddish brown. Chelicerae black. Cheliceral condyles orange brown. ALE nacelles black; POQ region black. Thin stripe of white appressed hair from AME to posterior region of PLE. Carapace dark reddish brown with black lines radiating from thoracic groove. Lighter yellow brown submarginal dashes, not forming continuous stripes. Dorsum of abdomen light brown with darker brown markings producing a mottled appearance. Cardiac region with brown lanceolate mark. Two or three faint darker chevrons posteriorly. Venter of abdomen with median area light brown, lateral areas cream to pale yellow, mottled with brown spots, not as distinct as in *T. helluo* and *T. georgicola.* Legs light yellow-brown to yellow, without darker bands as in female, except on leg IV where there are often dusky bands at proximal and distal ends of ventral surfaces. Scopulae on ventral surfaces of metatarsus and tarsus I. Labium and endites brown with yellow distal ends. Sternum brown.

Natural history.—Kaston (1981) reported that this species builds burrows from 5–8.5 inches [12.7–21.6 cm] deep. It comes out at night to hunt, but occasionally during the day it may be found under rocks or in pastures and the edges of woods. In Connecticut males occur in August to October, but females occur from early April to late September. The burrow is often surmounted by a turret of straw and twigs. According to Kaston (1981), mating occurs in the fall, and apparently at least two egg sacs are made each season. Adults probably live

two or three years. Large samples of *T. aspersa* from single localities were not often encountered, suggesting a more sedentary lifestyle than in *T. annexa, T. georgicola, and T. helluo*, but not unlike *T. grandis*. Because of its locally smaller numbers and secretive habits *T. aspersa* is the least common of those species that I have studied.

Distribution.—Ontario, Canada, and Massachusetts in the northeast, southward along the eastern seaboard to North Carolina, and then westward to Kentucky and Tennessee, and in the Midwest from Michigan to Iowa, and then southward to Missouri and Arkansas (Map 2).

Tigrosa georgicola (Walckenaer 1837)
new combination
Figs. 16–21, 42, Map 3, Table 3

Lycosa tarentuloides georgicola Walckenaer 1837:338.
Lycosa riparia Hentz 18*cola* Simon 1864:350.
Lycosa albopunctata Tullgren 1901:18.
Lycosa georgicola Chamberlin & Ivie 1944:143.
Allocosa georgicola Roewer 1955: 210.
Lycosa rivariola Bonnet 1957:2621 (replacement name).

Type material.—USA: *Georgia*, Burke County, specimen lost.

Remarks: Chamberlin (1908) listed *Lycosa georgicola* as an invalid name because the description was based upon the unpublished drawings of John Abbot. However, Chamberlin and Ivie (1944) resurrected the name *Lycosa georgicola* for this species and reproduced Abbot's original drawing #41 as the Type. There has been much controversy over this decision, but subsequent authors, including Roewer (1955), and Platnick (2011) recognized this name as valid. In my opinion Abbot's original drawing, which I have seen and photographed, is an illustration of this species. The only other close relative of *T. georgicola* in the Georgia region that is similar in color pattern is *T. helluo* and this species does not exhibit the banded legs seen in the illustration by Abbot. I have maintained the name *T. georgicola* for the sake of nomenclatural stability.

Other material examined.—USA: *Maryland*, Prince Georges Co., Patuxent Wildlife Refuge (39.07°N, 76.77°W), September 1962, no name, AMNH, 1♂, 3♀. *Virginia*, Fredericksburg [Independent City] (38.30°N, 7746°W), 29 August 1933, W. Ivie, AMNH, 1♂, 1♀; Halifax Co., (36.80°N, 78.88°W), 18 June 1935, no name, AMNH, 1♀ with egg sac; Sussex Co., Stony Creek (36.95°N, 77.40°W), 16 April 1951, Hoffman, AMNH, 1♀. *Tennessee*, Benton Co.(36.12°N, 88.05°W), 7 July 1952, T.J. Walker, Jr., AMNH, 1♀; Davidson Co., Nashville (36.17°N, 86.78°W), 7 May 1955, 1♀ with egg sac, 5 June 1955, 1♀, 20 June 1955, 1♂,1♀, 3 August 1955, 1♀, 22 September 1955, A.R. Laskey, AMNH, 1♂; Jackson Co., Scottsboro (36.22°N, 86.92°W), 1939, A.F. Archer, AMNH, 1♀, 2i; Obion Co., N of Samburg (36.38°N, 89.36°W), 9 July 1936, W.H. Parker, AMNH, 1♀; Roane Co., Kingston (35.88°N, 84.51°W), 12 July 1933, W. Ivie, AMNH, 2♀. *North Carolina*, Bladen Co., White Lake (34.64°N, 78.48°W), 26 March 1933, A.S. Pearse, MCZ, 1♀; Columbus Co., Lake Waccamaw (34.32°N, 78.50°W), 15 April 1933, A.M. Chickering, MCZ, 1♂, 1♀; Durham Co., Chapel Hill Boulevard at New Hope Creek (35.77°N, 79.04°W), 22 August 1963, J.W. Berry, MCZ, 1♂, Duke Forest, Durham (35.98°N, 78.90°W), 3 September 1932, 1♀, 7 July 1933, 1♀ with egg sac, 17–25 April 1935, 2♂, 1♀, 2 May 1938, A.M. Chickering, MCZ, 1♂, New Hope Valley, Durham (35.94°N, 78.95°W), 5 October 1935, A.M. Chickering, MCZ, 1♂; Mecklenburg Co., Davidson (39.50°N, 80.85°W), 20 September 1953, R.D. Barnes, AMNH, 1♀; Orange Co., Barbour Farm, St. Mary's Road (36.10°N, 79.04°W), 22 August 1933, J.W. Berry, MCZ, 1♂; Robeson Co., Maxton (34.74°N, 79.35°W), 15 May 1944, A.B. Klots, AMNH, 2♀. *South Carolina*, Horry Co., Myrtle Beach (33.69°N, 78.89°W), E. Mayr, 15 October 1940, AMNH, 1♂, 2♀, 1i. *Georgia*, Chattahoochee Co., Fort Benning (32.43°N, 84.94°W) 24 October 1943, 2♀, 1i, 6 December 1943, D.E. Beck, AMNH, 1♂; Liberty Co., St. Catherine's Island (31.66°N, 81.15°W) 23–29 April 1982, Rozen & Favreau, AMNH, 1♀; Rabun Co., Clayton (34.88°N, 83.40°W), 12 July 1960, S. & D. Mulaik, AMNH, 1♀. *Florida*, Alachua Co., 8 mi. [12.9 km] W of Gainesville (29.65°N, 82.32°W), 29 March 1957, W.J. Gertsch & R. Forster, AMNH, 1♂, 1♀, Newnan's Lake (29.65°N, 82.32°W), 13 June 1935, W. Ivie, AMNH, 1♀, 18 March 1938, 1♀, 13 April 1938, 1♀, 20 November 1938, W.J. Gertsch, AMNH, 1♀, West shore of Newnan's Lake, Gainesville (29.65°N, 82.32°W), 4 November 1932, 1♀, 22 January 1933, H.K. Wallace, AMNH, 3♂, 21 December 1962, W. Ivie, AMNH, 2♂, Sugarfoot Hammock (29.66°N, 82.30°W), 19 March 1938, W.J. Gertsch, AMNH, 10♂, 15♀, 1 egg sac, 3i; Broward Co, Palm Forest (26.04°N, 80.31°W), 1938, D. Cottam, AMNH, 2♀; Charlotte Co., Punta Gorda (26.93°N, 82.05°W), February 1941, Ramstadt, AMNH, 1♀; Collier Co., Tamiami Trail at Turner River (25.89°N, 81.26°), 18 December 1962, W. Ivie, AMNH, 1♀; Dade Co., Miami (25.79°N, 80.23°W), H.K. Wallace, 1 February 1947, 1♂; Highlands Co., Highlands Hammock State Park (27.46°N, 81.55°W), 24 March 1938, W.J. Gertsch, AMNH, 3♂, 9♀, 6i, 20 April 1973, A.R. Brady, HCC, 1♀; Jackson Co., Spring Lake (30.70°N, 85.29°W), 10 July 1981, W.H. Cross, MSST, 1♂, 3♀; Leon Co., Tall Timbers Research Station (30.49°N, 84.19°W), 9 June 1968, A.R. Brady, MCZ, 1♀; Liberty Co., Blountstown (30.44°N, 85.04°W), 17 April 1938, W.J. Gertsch, AMNH, 1♀, Torreya State Park (30.57°N, 84.95°W), 16 April 1938, W.J. Gertsch AMNH, 2♂, 1♀ with egg sac, 31 March 1964, H.W. Levi, 1♀, 28 March 1965, A.R. Brady, HCC, 1♀, 12 May 1997, C.T. Reif & J.M. Ziter, HCC, 1♀, 13 May 1997, A.R. Brady, HCC, 1♀, 22 June 1997, A.R. Brady, 1♀, 27 December 1997, A. Wyatt, HCC, 1♀; Monroe Co., Key West (24.55°N, 81.80°W), 5 February 1967, no name, AMNH, 1♀; Okeechobee Co., Okeechobee (27.25°N, 80.83°W), 29 March 1938, W.J. Gertsch, AMNH, 1♀; Putnam Co., Welaka Reserve (29.48°N, 81.67°W), 5 May 1973, A. Jung, HCC, 1♀; Sarasota Co., Myakka River State Park (30.49°N, 84.19°W), 7 Mareh 1963, H.& L. Levi, MCZ, 1♀; Seminole Co., Geneva (28.74°N, 81.11°W), 11 April 1938, W.J. Gertsch, AMNH, 3♂; Volusia Co., Deland (29.03°N, 81.30°W), F.E. Lutz, 25 May 1939, AMNH, 1♀, Enterprise (28.87°N, 81.27°W), no date, N. Banks, MCZ, 1♀, 1i. *Alabama*, Baldwin Co., Fish River (30.45°N, 87.80°W), 14 July 1930, S. Creighton, MCZ, 2♀; Coosa Co., Hatchet Creek (32.87°N, 86.32°W), June 1940, A.F. Archer, AMNH, 5i; Dallas Co., Selma (32.41°N, 41.87°W), no date, R.V. Chamberlin, MCZ, 1♀; Escambia Co., Flomaton (31.00°N, 87.26°W), August 1903, A P. Morse, MCZ, 1♀; Humphreys Co., (33.17°N, 90.53°W), 10–30 November 1937, no name, AMNH, 1♂; Jackson Co., Guess' Creek (34.75°N, 86.23°W),

July 1940, A.F. Archer, AMNH, 1♀, Scottsboro (34.67°N, 86.03°W), 1939, A.F. Archer, AMNH, 1♀, 1i; Lawrence Co., Blaek Warrior National Forest (34.56°N, 87.30°W), June 1939, A.F. Archer, AMNH, 1♀; Lee Co., Auburn (32.61°N, 85.48°W), R.V. Chamberlin, no date, MCZ, 1♀. *Mississippi*, Forest Co., Camp Shelby, Hattiesburg (31.18°N 89.20°W), October–November 1945, C. D. Michener, AMNH, 1♀; Jackson Co., Ocean Springs (31.0°N, 86.86°W), 2 August 1961, G. Gunter, MCZ, 1♀, (86.03°W), 1939, A.F. Archer, AMNH, 1♀, 1i, Vancleave (30.54°N, 88.69°W), no name, 10–30 November 1937, AMNH, 1♂; Lafayette Co., Oxford (34.36°N, 89.52°W), 20 July 1991, A.R. Brady, HCC, 1♂; Lincoln Co., 19 July 1910, R.V. Chamberlin, AMNH, 1♀; Madison Co., 1 mi. [1.6 km] N of Ridgeland (32.43°N, 90.14°W), 3 October 1982, T.C. Lockley, HCC, 1♂; Mobile Co., (34.57°N, 87.30°W), no date, H.P. Loding, MCZ, 1♀; Oktibbeha Co., Craig Springs (33.32°N, 88.92°W), 1 October 1979, 1♂, 3 October 1979, 1♂, 29 October 1979, G. L. Snodgrass, MSST, 3♂, Mississippi State University (33.45°N, 88.79°W), 3 April 1904, no name, AMNH, 1♀, B. Booth, 22 July 1983, MSST, 1♀, Starkville (33.47°N, 88.81°W), 5 June 1980, 1♀, 2 July 1980, 1♀, 3 July 1980, 1♀, 26 September 1980, 2♂, 1♀, 10 October 1980, 2♂, 1i, 2 August 1982, W.H. Cross, MSST, 2♂, 1♀, 18 August 1982, P.R. Miller, MSST, 2i, 25–27 Aug.1982, T.C. Lockley, HCC, 1♀, 29 April–2 May 1983, 1♂, 2–4 May 1983, 1♀, 1–13 May 1983, 1♀, 6–8 June 1983, 2♂, 10–14 June 1983, 1♂, 2♀, 15–17 June 1983, 1♂, 28 June–1 July 1983, 2♂, 1–6 July 1983, 1♂, 11–13 July 1983, 1♀, 21–25 July 1983, 1♀ with egg sac, 22 July 1983, 1♀, 24 August 1983, 1♀, 23–25 August 1983, T.C. Lockley, HCC, 1♀; Panola Co., 11 mi. [17.7 km] SW of Bates (30.72°N, 88.178°W), 1 August 1979, W.H. Cross, MSST, 1♂; Perry Co. (31.17°N, 89.02°W), 24 March 1938, 1♀, 1–11 May 1938, AMNH, S.C. Bishop, 2♀; Pontotoc Co., 1 mi. [1.6 km] SE of Ecru (34.35°N, 89.03°W), 5 June 1980, 1♀, 6 June 1980, 2♂, 17 June 1980, 1♀, 19 June 1980, 2♂, 2 July 1980, 1♀, 3 July 1980, 1♀, 1 August 1980, 1♀, 14 August 1980, 26 September 1980, 2♂, 1♀, 10 October 1980, 2♂, 1i, W.H. Cross, MSST, 1♀, 6 June 1980, P.R. Miller, MSST, 1♂, 1i; Rankin Co., Thompson Field (32.32°N, 89.99°W), 1–3 October 1982, 2♂, 7–9 January 1983, 1♂, 21 April 1983, 1♂, 1i, 9–11 May 1983, 1♂, 13–15 May 1983, 2♂, 8–10 July 1983, 1♂, 7 August 1983, 1♂, 9–11 September 1983, 1♀ with young, T.C. Lockley, HCC, 1♀; Scott Co., Roosevelt State Park (32.31°N, 89.69°W), 26 August 1940, S. & D. Mulaik, AMNH, 1♂; Washington Co., Leland (90.90°N, 33.40°W), 19 June 1982, 1♀, 27 July 1982, 1♂, 17–20 December 1982, 1♂, T.C. Lockley, HCC, 1♂,1i, 15–18 July 1983, 1♀, T.C. Lockley, HCC, 5 mi. [8.0 km] SSE of Leland (90.90°N, 33.40°W), 19–23 August 1982, 1♂, 25–27 August 1982, HCC, T.C. Lockley, HCC, 1♂, 2 mi. [3.2 km] N of Stoneville (33.42°N, 90.92°W), 31 May–2 June 1982, 2♂, 19 August 1982, 1♀, 13–16 August 1982, 1i, 18–20 August 1982, 1i, 23–25 August 1982, 1♀, 25–27 August 1982, 1♀, 27–30 August 1982, 1♀, 8–10 September 1982, 1♂, 15–17 September 1982, 2♂, 24–27 September 1982, 1♂, 27–29 September 1982, 3♂, 29 September–1 October 1982, 3♂, 3 October 1982, 1♂, 1–4 October 1982, 6♂, 20–22 October, 1♂, 1♀, 29 October–1 November, 1i, 1982, 29 November–1 December 1982, 1♂, 4 April 1983, 1i, 30 April–2 May 1983, 1♂, 7 May 1983, 2♂, 6–9 May 1983, 1♂, 1i, 11–13 May 1983, 1♂, 1♀, 31 May–2 June 1983, 1♂, 3–6 June 1983, 4♂, 3i, 6–8 June, 1983, 5♂, 8–10 June, 1983, 1♂, 10–14 June 1983, 1♂, 2♀, 15–17 June 1983, 1♂, 20–22 June 1983, 1♂, 26–28 June 1983, 3♂, 28 June–1 July 1983, 2♂, 1–6 July 1983, 1♂, 11–13 July, 1♀ with young, 13–15 July, 2♂, 21–25 July 1983, 1♀ with egg sac, 25–28 July 1983, 1♂, 27 July 1983, 1♀, 28 July–1 August 1983, 2♂, 1 August 1983, 1♀, 12–13 August 1983, 1♂, 15 August 1983, 1 ♀, 16 August 1983, 1♀, 18–21 August, 1♂, 24 August 1983, 1♀, 1 September 1983, T.C. Lockley, HCC, 1♀; *Louisiana*, Ascension Par., Gonzales (39.24°N, 90.92°W), 31 August 1940, S. & D. Mulaik, AMNH, 2♀, 4i; Catahoula Par., Camp Plauche (31.24°N, 92.15°W), 4 April 1944, D.E. Beck, AMNH, 1♀ with egg sac; East Baton Rogue Par., (30.97°N, 91.52°W), July 1955, no name, AMNH, 1♀; Madison Par., Tallulah (32.41°N, 91.19°W), L.I. Davis, 6 June 1930, AMNH, 2♂, February 1935, J.W.F., AMNH, 1♀; Orleans Par., New Orleans (29.96°N, 90.07°W), 16 January 1920, H.E. Hubert, HCC, 3♀, 8 May 1945, W. Spector, AMNH, 3♀, 14 October 1961, G. Walker, AMNH, 1♂, ♀. *Arkansas*, Lawrence Co., Imboden (36.20°N, 91.17°W), 1935, B.C. Marshall, AMNH, 1♂, 4♀, 1i. *Kansas*, Cowley Co., Winfield (37.24°N, 97.00°W), no date, no name, AMNH, 3♀. *Texas*, Austin Co., Sealy (29.78°N, 96.16°W), 19 April 1942, O. Sanders, AMNH, 1♀; Bastrop Co., Bastrop State Park (30.11°N, 97.26°W), 25 October 1958, A.R. Brady, MCZ, 1♀, 1i; Brazos Co., College Station (30.63°N, 96.33°W), June 1939, H. Menusan, AMNH, 1♀, 2.4 mi. [3.86 km] S of Old Ocean (29.08°N, 95.75°W), 23 December 1961, R.O. Albert, MCZ, 1♀; Caldwell Co., Luling (29.68°N, 97.65°W), 11 April 1948, A. Flury, AMNH, 5♀, 1 egg sac; Comal Co., Honey Creek Ranch (29.81°N, 98.22°W), 29 October 1983, T.C. Lockley, HCC, 1♀; Gonzales Co., Palmetto State Park (29.96°N, 96.16°W), W. F. Blair & K. Baker, AMNH, 1♀, 2 July 1979, A.R. Brady, M.A. Brady & W. Webb, HCC, 3♀, 8i, 3 July 1979, M.A. Brady, HCC, 2♀, 1i, 11–12 July 1993, A.R. Brady, HCC, 1♂, 1 ♀ with young; Grayson Co., Sherman (33.64°N, 96.61°W), 4 July 1964, K.W. Haller, AMNH, 1♀, 17 May 1965, K.W. Haller, AMNH, 1♀; Hardin Co., Saratoga (30.28°N, 94.53°W), 22 November 1958, A.R. Brady, MCZ, 3♀, 1i, 5 mi. [8.0 km] SW of Saratoga (30.28°N, 94.53°W), 2 May 1948, D.L. Jamison, AMNH, 1♀; Harris Co., Houston (29.76°N, 95.37°W), 22 Mareh 1936, no name, AMNH, 1♀, Spring Creek near Houston (29.87°N, 95.66°W), 27 March 1936, no name, AMNH, 1♀; Harrison Co., Caddo Lake State Park (32.68°N, 94.18°W), 22 August 1940, S. & D. Mulaik, AMNH, 1♀; Hays Co., (30.05°N, 98.00°W), 15 April 1939, D. & S. Mulaik, AMNH, 1♂, 3♀, San Marcos (29.88°N, 97.94°W) 22 April 1935, Vogelsang, MCZ, 1♀; Jefferson Co., Beaumont (30.09°N, 94.10°W), April–June 1946, E.D. Palmer, MCZ, 2♀; Jim Wells Co., Alice (27.75°N, 98.07°W), 1–18 June 1961, R.O. Albert, MCZ, 1♀; Karnes Co., San Antonio River near Runge (28.88°N, 97.21°W), 1959, J.C. Bequaert, MCZ, 1♀; Kerr Co., Raven Ranch (30.09°N, 99.46°W), August 1934, D. Mulaik, AMNH, 1♀; Leon Co., Birch Creek near Marquez (31.30°N, 96.32°W), 26 October 1958, A.R. Brady, MCZ, 1♂, 3♀; Nacogdoches Co., Nacogdoches (31.60°N, 94.66°W), 11 August 1964, J. & W. Ivie, AMNH, 1♀; Panola Co., Sabine River, NE of Carthage (32.16°N, 94.34°W), 12 April 1963, W.J. Gertsch, W. Ivie, AMNH, 1♀ with egg sac; San Patricio Co., 8 mi. [12.9 km] SE of Sinton (28.04°N, 97.51°W), 28 April 1960, 2♂, 1i, 26 May 1960, H.E. Laughlin, AMNH, 2♂; Travis Co., Zilker Park,

Austin (30.26°N, 97.77°W), 11 March 1946, A. Flury, AMNH, 1♀.

Diagnosis.—*Tigrosa georgicola* (Figs. 16, 17) most closely resembles *T. helluo* (Figs. 28, 29) in dorsal color pattern, but it tends to be larger than *T. helluo*, for example in average body length *T. georgicola* measures 21 mm, but *T. helluo* measures 17 mm. Dimensions of the eye rows and length of legs also illustrate the size difference (compare Table 3 with Table 5). In addition the legs of *T. georgicola* have dusky bands or markings. The venter of *T. georgicola* (Fig. 42) consists of longitudinal rows of dark spots or dashes that produce a mueh darker appearance than in the spotted venter of *T. helluo* (Fig. 41). Although *T. georgicola* and *T. aspersa* are similar in size, the median stripe in female *T. georgicola* extends from the PME region to the posterior declivity (Fig. 16), while in *T. aspersa* it is restricted to the eye region (Fig. 10). In male *T. georgicola* the submarginal stripes are clearly visible and extend posteriorly from the cephalic region to the posterior declivity (Fig. 17), but in *T. aspersa* the submarginal stripes are represented by a series of disconnected shorter segments (Fig. 11). In *T. georgicola* the MA of the palpus (Fig. 20) is more strongly developed than in *T. aspersa* (Fig. 12), and the palea in *T. georgicola* is squarer in retrolateral view (Fig. 22) than in *T. aspersa,* where it is smaller and rectangular (Fig. 13).

Remarks.—It is difficult to know why Roewer (1955) placed *T. georgicola* in the genus *Allocosa* (Banks 1900). He did not describe any essential characteristics that *T. georgicola* might share with *Allocosa*, nor did he offer any significant diagnosis of *T. georgicola. Tigrosa georgicola* is much larger than species of *Allocosa* in North America. Body morphology and eye arrangement are different in the two genera. There are also differences with respect to choice of habitat and foraging behavior between representatives of these two genera. Most importantly the characteristics of the male palpus and female genitalia differ significantly between *Tigrosa* and species of *Allocosa.* On the other hand *T. georgicola* is very similar to other species of *Tigrosa* with respeet to the taxonomic characteristics explored in this investigation.

Occasionally in *T. georgicola* the dark color covers much of the ventral surface and led Chamberlin & Ivie (1944) to describe the melanic form as *Lycosa wallacei.* Although they indicate differences in the epigyna of these two "species", they do not elaborate upon the differences. The males were described as "essentially the same". In *Lycosa wallacei* the legs often lack distinct annulations, adding to the color differences, but I am unable to find consistent genitalie differences between those with a black venter and the more common venter described above. Whether one or two species is involved remains to be determined by closer scrutiny of populations by arachnologists in the field.

Color pattern.—*Female:* Dorsal pattern illustrated in Fig. 16. Face reddish brown to dark brown, lighter color lateral to AME row. Chelicerae black, condyles dark reddish brown. Carapace orange brown with dark brown to black lines radiating from thoracic groove. Eye region dark brown to black. Median longitudinal pale-yellow stripe from AME row to posterior declivity, clothed with white to cream colored hair, between PME to PLE. Submarginal pale yellow to orange brown submarginal stripes with uneven margins. In some specimens the submarginal lighter stripes essentially reach the edge of the carapace, separated from the edge by narrow darker margins. Dorsum of the abdomen uniformly brown with dark brown cardiac mark accented by lighter color. Only a very faint suggestion of chevrons or none at all posterior to cardiac area (Fig. 16). Venter of abdomen with three central dark stripes originating at the epigastric furrow and converging at base of spinnerets. Laterally there are dark irregular spots against a lighter brownish yellow background (Fig. 42). Legs light yellow orange to pale yellow brown without distinct darker bands or with three gray bands on dorsal surfaces of femora III and IV. Legs lighter yellow to pale orange ventrally. Labium dark brown to black with yellowish distal ends. Sternum as well as ventral coxae dark brown to black.

Male. Pattern illustrated in Fig. 17. Face brown below anterior eye row; lateral area (cheeks) yellow and darker brown above. Eye region blaek. Thin white stripe from AME, running between PME. Chelicerae dark brown to black. Carapace orange brown with broad pale yellow marginal stripes with dark outer edges. Thin median pale yellow stripe from PME to posterior declivity, clothed with white hair between AME to PME. Dorsum of abdomen light brown, mottled with darker spots and suffused with white hair. Distinct brownish orange lanceolate shaped cardiac mark bordered by dark brown (Fig. 17). Venter of abdomen pale brown (beige), lighter cream to yellow above epigastric furrow. Three longitudinal rows of darker brown spots, the lateral rows beginning at the corners of the epigastric furrow and converging with the median row in front of the spinnerets. Dark brown spots against cream to light brown in lateral areas (similar to the female in Fig. 42). Legs yellow without dusky bands as in female. Ventral surfaces pale yellow to cream. Labium and endites light brown with yellow distal ends. Sternum light brown with pale yellow dash posterior to labium.

Natural history.—*Tigrosa georgicola* occurs widely in the southeastern United States, where it is found in deciduous woods, often under logs during the day and hunting over leaf litter at night. It occasionally occurs in short herbaceous vegetation at the edge of woods. This species overlaps in distribution with *T. helluo* and *T. annexa.* The former species is found in wetter habitats, such as swamps and bogs, and the latter is found in drier habitats, such as grass or cotton fields. Although *T. aspersa* overlaps in distribution with these three species, it apparently stays eloser to its burrow, not wandering far from its retreat in search of food.

Tigrosa grandis (Banks 1894)
new combination
Figs. 22–27, 43, Map 4, Table 4

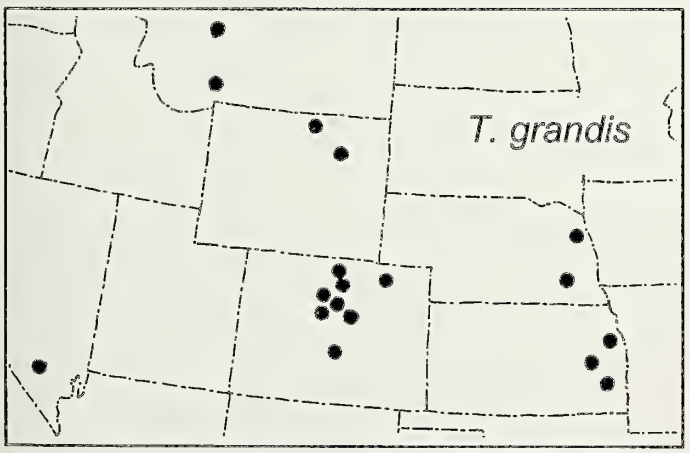
Lycosa grandis Banks 1894:49; Chamberlin 1908:229.
Lycosa permunda Chamberlin 1904:286; Chamberlin 1908:233.
Geolycosa grandis Roewer 1955:244; Platnick 2011.
Hogna permunda Roewer 1955:259; Platnick 2011.
Hogna grandis Slowik & Cushing 2009:261; Platnick 2011.

Type material.—*Holotype:* USA: *Colorado*, Larimer County, Fort Collins (40.35°N, 105.05°W), elev. 1525 m, no date, ♂, MCZ. Examined. *Holotype male* and *paratype female: Lycosa permunda* Chamberlin 1904. USA: *Kansas,* no date. Unable to locate specimens.

Table 4.—Mean and range of ten females and ten males of *Tigrosa grandis* from Colorado.

	Mean (range)		Mean (range)
Females			
Anterior eye row	1.85 (1.6–2.1)	Femur I	7.21 (5.6–8.8)
PME width	1.96 (1.6–2.2)	Patella-Tibia I	9.20 (6.8–10.8)
PLE width	2.67 (2.1–3.1)	Metatarsus I	4.95 (3.7–5.9)
POQ length	1.83 (1.5–2.2)	Tarsus I	3.17 (2.5–3.7)
Car. width at PLE	5.52 (4.1–6.9)	Total length I	24.39 (18.6–28.7)
Carapace width	8.39 (6.4–10.2)	Femur IV	8.09 (6.3–9.7)
Carapace length	11.12 (8.6–13.2	Patella-Tibia IV	9.75 (7.6–11.3)
Body length	22.48 (17.6–28.2)	Metatarsus IV	8.10 (7.2–9.6)
Patella-Tibia II	8.33 (6.3–9.8)	Tarsus IV	3.71 (2.9–4.1)
Patella-Tibia III	7.42 (5.6–8.8)	Total length IV	29.65 (22.6–34.3)
Males			
Anterior eye row	1.87 (1.7–2.1)	Femur I	8.72 (7.8–9.4)
PME width	2.08 (1.9–2.2)	Patella-Tibia I	11.20 (10.2–12.8)
PLE width	2.79 (2.5–3.0)	Metatarsus I	6.93 (6.4–7.8)
POQ length	1.98 (1.7–2.3)	Tarsus I	4.28 (4.0–5.1)
Car. width at PLE	5.47 (4.5.–6.0)	Total length I	31.26 (28.6–35.1)
Carapace width	9.11 (7.8–10.4)	Femur IV	9.67 (8.5–10.6)
Carapace length	11.89 (10.4–13.3)	Patella-Tibia IV	11.62 (10.5–13.0)
Body length	22.22 (20.1–25.3)	Metatarsus IV	10.27 (9.3–12.0)
Patella-Tibia II	10.16 (9.0–11.6)	Tarsus IV	4.43 (4.1–4.9)
Patella-Tibia III	8.92 (8.2–10.2)	Total Length IV	35.99 (32.6–40.0)

Other material examined.—*Nebraska*, Lincoln, W. Clanton (40.81°N, 96.68°W), 28 August 1923, no name, AMNH, 1♂. *Kansas*, Franklin Co. (38.62°N, 95.31°W), 1935, AMNH, 1♂. Linn Co., Parker (38.33°N, 94.99°W), 4 October 1965, B. Reeves, AMNH, 1♀. Wyandotte Co., Mission Grade School, 2 mi. [3.2 km] N of Bonner Springs (39.16°N, 94.83°W), 29 September 1977, R. Hugging, DMNS, 1♂. *Montana*, Cascade Co., Great Falls (47.50°N, 111.30°W), no name, 15 August 1976, AMNH, 1♂, 1♀; Gallatin Co., Bozeman (45.68°N, 111.05°W), no date, H.B. Mills, AMNH, 1♀ with young). *Wyoming*, Campbell Co., Gillette, (44.29°N, 105.50°W), June 2005, no name, DMNS, 1♂, 1♀; *Sheridan Co.*, Sheridan (44.80°N, 106.96°W), 29 June 1949, D. G. Penning, AMNH, 1♀ with egg sac. *Colorado*, Arapahoe Co., Aurora (39.65°N, 104.75°W), 20 August 2001, B. Shipley, DMNS, 1♀, 2 mi. [3.2 km] E of Marshall (39.96°N, 105.23W), 16 April 1961, B. Vogel, DMNS, 1♀, I-70, 0.5 mi. [0.80 km] N of Elberta Co. Line (39.58°N, 104.02°W), 30 August 2004, H. Guarisco, DMNS, 1♀; Boulder Co., (40.01°N, 105.27°W), 19 May 1912, Betts, AMNH, 1♀ with egg sac; Denver Co., Denver (39.74°N, 104.98°W), 8 August 1971, Lamore, AMNH, 1♀, Washington Bay in Denver near Southwest Plaza (39.83°N, 105.20°W), October 1998, D.M. Endricks, DMNS, 1♀; El Paso Co., Billerest Terrace (38.88°N, 104.76W), 16 July 2001, A. Broughton, DMNS, 1♀; Jefferson Co., Arvada (39.80°N, 105.09°W), 8 July 1933, C.H. Moss, AMNH, 1♀ with egg sac, 9797 West Ohio Avenue, Lakewood (39.70°N, 106.11°W), August 2001, E. House, DMNS, 1♂; *Larimer Co.*, 1756 Haase Court, Berthoud (40.31°N, 105.81°W), 18–20 August 1999, P. Phillips, DMNS, 1♂, 2120 Bridgefield Lane, 24205 Colorado Ave., Loveland (40.37°N, 105.08°W), 30 July 1999, D. Goldade, 1♀, 7894 Little Fox Lane, Wellington (40.70°N, 105.00°W), 14 September 2000, M. Payew, DMNS, 1♀, Dixon Reservoir (40.55°N, 105.14°W), 24 May 2000, D. Chlebown, DMNS, 1♀, Environmental Learning Center (40.57°N, 105.01°W), 25 September 1999, J.M. Diez, DMNS, 1♀, Fort Collins (40.56°N, 105.10°W), 29 August 2004, J. Enstrom, DMNS, 1♀, Ft. Collins (40.58°N, 105.11°W), 10 August 1970, 1♂, 4 January 1971, 1♀, 30 August 1973, 1♀, 18 September 1973, 1♀, 1 September 1977, 1♂, 1♀, 15 July 1980, 1♀, 24 November 1980, 1♂, 4 March 1985, W.D. Frank, DMNS, 1♂, 22 October 1982, D. Clarkson, DMNS, 1♀, 9 August 1987, D. Johnson, DMNS, 1♂, 13 June 1989, 7 August 1990, Kilburn, DMNS, 1♂, B. Holter, DMNS, 1♀, 1 September 1990, no name, 1♀, 23 September 2001, L. Sander, DMNS, 1♂, Loveland (40.40°N, 105.11°W), 10 October 1967, 1♀, W.D. Frank, DMNS, 1♀, DMNS, 1♀; Weld Co., Bones Galore Paleo Site, Pawnee National Grassland (40.73°N, 103.80°W), 15 August 2001, T. Hiester, DMNS, 1♂. *Nevada*, Nye Co., Mercury Test Site (36.66°N, 116.00°W), 18 June 1963, G. Hayward, AMNH, 2♂. *Montana*, Cascade Co., Great Falls (47.50°N, 111.30°W), 15 August 1976, B. Cutler, AMNH, 1♂, 1♀.



Map 4.—Distribution Map of *Tigrosa grandis*.

Diagnosis.—*Tigrosa graudis* and *T. aspersa* are the two largest species of *Tigrosa*. Most specimens of *Tigrosa graudis* were collected west of the 100th meridian (Map 4), and most specimens of *T. aspersa* were collected east of the 100th meridian (Map 2). In female *T. graudis* the median longitudinal light stripe originates in the AME region and extends to the posterior declivity of the carapace (Fig. 22), while in *T. aspersa* it is limited to the cephalic region (Fig. 10). In female *T. graudis* the LP of the median septum of the epigynum (Fig. 26) is narrower than in *T. aspersa* (Fig. 15) and the TP in *T. aspersa* is more spade-shaped than T-shaped. In *Tigrosa graudis* the LP is shorter and the TP is thicker than in *T. helluo* (Fig. 33). The atrium of the epigynum in *T. graudis* is not as pronounced as in *T. georgicola* (Fig. 18), and the TP is thicker. The bilobed terminal chamber of the spermathecae of *T. graudis* (Fig. 27) distinguishes it from females of *T. aspersa* (Fig. 14) and *T. georgicola* (Fig. 19). Male *T. graudis* (Figs. 24, 25) have a shorter median apophysis (MA) than *T. helluo* (Figs. 30, 31) and *T. georgicola* (Figs. 20, 21). The submarginal stripes on the carapace in male *T. graudis* are continuous from the posterior cephalic region to the posterior declivity (Fig. 23), while in *T. aspersa* the stripes consist of disconnected segments (Fig. 11). Also, the dorsum of the abdomen in *T. graudis* is uniformly brown except for the dark cardiac mark and a series of darker chevrons posterior to the cardiac area (Fig. 23), while in *T. aspersa* the dorsum is mottled in appearance without distinct chevrons posterior to the cardiac area (Fig. 11).

Color pattern.—*Female:* Dorsal pattern illustrated in Fig. 22. Face dark brown with eye nacelles black. White appressed hair lateral to AME row in cheek area. Chelicerae black, hirsute. Thin yellow line from AME extending between PME with width one-half the diameter of PME. Carapace dark reddish brown with black lines radiating from thoracic groove. Narrow median longitudinal stripe, yellow to tan in color, extending from AME region to posterior declivity, slightly wider as it surrounds thoracic groove. Lighter tan uneven or scalloped submarginal stripes, less conspicuous than median longitudinal stripe. Dorsum of abdomen dark brown with black, oblong rectangular mark surrounding cardiac area. Three to four darker chevrons posterior to cardiac region, accented with white and with white dots at edges. Venter with central area cream to light brown or tan from epigastric furrow, tapering to base of spinnerets. Epigastric region cream to yellow. Lateral areas mottled with dark brown spots against a lighter background (Fig. 43). Legs tan to light brown, darker than in male. Labium dark reddish brown with yellow distal ends. Sternum dark reddish brown with median yellow dash or short line.

Male. Pattern illustrated in Fig. 23. Face dark reddish brown with covering of lighter appressed hair on clypeus. Chelicerae dark reddish brown with black condyles. Narrow yellow line from AME running between PME. Carapace dark reddish brown with black lines radiating from thoracic groove to submarginal stripes. Narrow median yellow line from PME to posterior declivity, widest at thoracic groove. Pale scalloped submarginal stripes present, but not as sharply delineated as in female. Dorsum of abdomen mottled brown and pale yellow with brown lanceolate mark in cardiac region. Three or four darker chevrons on posterior half. Lateral areas lighter in color. Venter of abdomen cream to pale yellow without darker spots or stripes. Legs light brown on dorsal surfaces, lighter brown to yellow ventrally. Labium dark brown to black with yellow distal ends. Sternum light brown with faint median longitudinal stripe. For slightly different and more detailed description see Slowik & Cushing (2009).

Natural history.—Slowik has personally observed *T. grandis* occupying burrows and wandering at night in search of prey (Slowik & Cushing 2009). This behavior is not unlike that of *Hogna carolinensis* (Walckenaer 1805). The burrows ranged from 2–4 inches [5.1–10.2 cm] in depth, but were not surmounted by turrets, as is often the case with *H. carolinensis*. Deeper burrows were vertical with a small cavity at the base, while shallower burrows tended to be more horizontal in position.

Distribution.—*Tigrosa graudis* has been found from eastern Kansas southwestward to Nevada and northwestward to Montana (Map 4). Slowik & Cushing (2009) report collecting *T. graudis* from the grasslands east of the Rocky Mountains in Wyoming and Colorado and west into the San Luis Valley of Colorado. Misidentification of specimens of *T. helluo* from Nebraska as *T. grandis* has created discrepancies in the distribution records for these two species. Apparently neither *T. helluo* nor *T. aspersa*, which occur more eastward than *T. graudis*, are found in Wyoming or Colorado. See Slowik and Cushing (2009) for a more detailed discussion of this situation.

Tigrosa helluo (Walckenaer 1837)
new combination
Figs. 28–33, 41, Map 5, Table 5

Lycosa helluo Walckenaer 1837:337; Chamberlin 1908:226; Comstock 1913:633, 1940:645; Muma 1943:46; Kaston 1948:327; Griswold 1993:3.
Lycosa sayi Walckenaer 1837:337.
Lycosa babiugtoui Blackwall 1846:30.
Tarentula vafra C. L. Koch 1847:135.
Leimouia helluo Simon 1864:351.
Leimouia sayi Simon 1864:351.
Trochosa helvipes Keyserling 1877:659.
Lycosa uidicola Emerton 1885:482, 1902:69; Montgomery 1902:559. *Lycosa crudelis* Banks 1892:66.
Hogna helluo Roewer 1955:258; Dondale & Redner 1990:51; Bennett 1992:8; Paquin & Dupérré 2003:161; Slowik & Cushing 2009:263.

Type material.—Holotype: USA, *New York*, specimen lost.

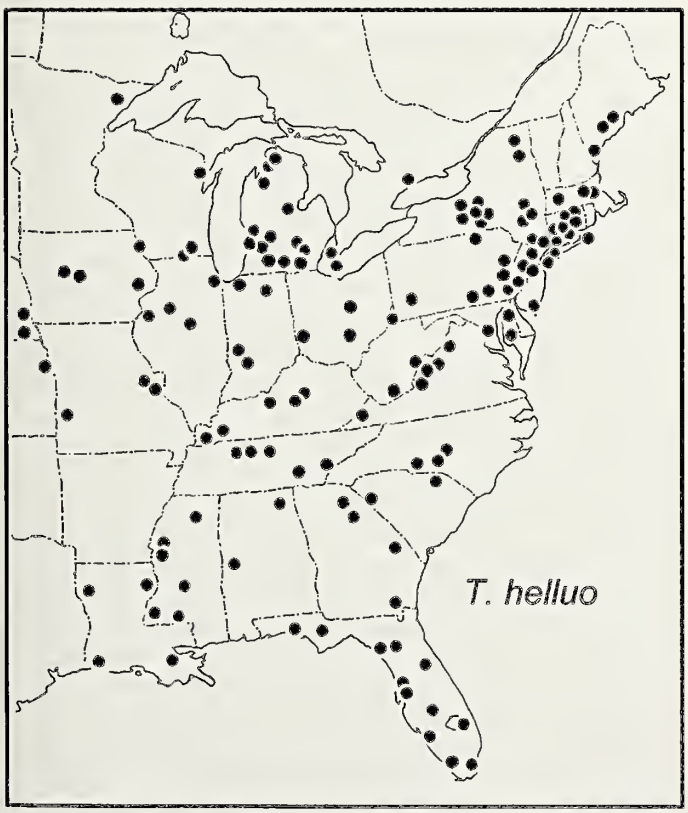
Remarks.—Walckenaer (1837) provided a brief written description of *Lycosa helluo*, presumably based upon specimens that he had examined. Unfortunately no one has been able to locate these specimens. Chamberlin (1908) based his determination of *Lycosa helluo* upon Walckenaer's description. Chamberlin (1908) then provided a lengthy description and illustrations of the epigynum and male palpus of what he called *Lycosa helluo* Walckenaer. This diagnosis established the identity of *Lycosa helluo* and was recognized by subsequent North American arachnologists, particularly Comstock (1913) and Kaston (1948, 1981). In my opinion Chamberlin's description is diagnostic and Walckenaer's name for this species should be retained for the sake of nomenclatural stability.

Other material examined.—CANADA: *Ontario*, Essex Co., Pelee Island (41.77N°, 82.69°W), 4–16 June 1950, W. Ivie & T.B. Kurata, AMNH, 3♀, Point Pelee National Park (41.95°N, 82.51°W), 5 September 1953, D.E. Johnston, AMNH, 1♀; Highland Creek near Toronto (43.78°N, W79.17°W) 24 July

Table 5.—Mean and range of ten females and ten males of *Tigrosa helluo* from New York.

	Mean (range)		Mean (range)
Females			
Anterior eye row	1.49 (1.32–1.7)	Femur I	5.76 (4.8–6.7)
PME width	1.66 (1.6–1.9)	Patella-Tibia I	7.29 (6.0–8.5)
PLE width	2.17 (1.9–2.5)	Metatarsus I	4.03 (3.3–4.7)
POQ length	1.51 (1.4–1.7)	Tarsus I	2.70 (2.3–3.1)
Car. width at PLE	4.00 (3.3–4.8)	Total length I	19.78 (16.6–22.9)
Carapace width	6.42 (5.3–7.6)	Femur IV	6.56 (5.5–7.7)
Carapace length	8.39 (6.9–9.7)	Patella-Tibia IV	7.93 (6.7–9.0)
Body length	17.46 (14.9–21.5)	Metatarsus IV	6.92 (6.0–8.0)
Patella-Tibia II	6.57 (5.3–7.7)	Tarsus IV	3.31 (2.7–3.7)
Patella-Tibia III	5.83 (4.8–6.7)	Total length IV	24.71 (21.0–28.5)
Males			
Anterior eye row	1.09 (.96–1.2)	Femur I	4.92 (4.0–5.7)
PME width	1.24 (1.1–1.4)	Patella-Tibia I	6.50 (5.3–7.7)
PLE width	1.60 (1.4–1.8)	Metatarsus I	4.42 (3.5–5.2)
POQ length	1.15 (1.0–1.3)	Tarsus I	2.79 (2.3–3.2)
Car. width at PLE	2.51 (2.1–2.9)	Total length I	18.63 (15.0–21.8)
Carapace width	4.43 (3.7–5.2)	Femur IV	5.55 (4.7–6.5)
Carapace length	5.80 (4.9–6.9)	Patella-Tibia IV	6.77 (5.5–7.8)
Body length	11.25 (9.6–12.9)	Metatarsus IV	6.40 (5.2–7.6)
Patella-Tibia II	5.71 (4.7–6.7)	Tarsus IV	3.10 (2.5–3.7)
Patella-Tibia III	4.85 (3.9–5.7)	Total Length IV	21.81 (17.8–25.5)

1943, S. Herrod, AMNH, 1♂. USA: *Maine*, Lincoln Co., no specific locality (44.06°N, 69.54°W), 10–20 July 1948, D.J. Borror, AMNH, 1♀, Waldoboro (43.43°N, 70.66°W), 1971, Mrs. E.M. Lloyd, MCZ, 1♀; York Co., Wells (43.15°N, 70.67°W), 12 August 1933, W. Ivie, AMNH, 4♂. *Massachusetts*, Hampshire Co., Amherst (42.38°N, 72.52°W), 4 October



Map 5.—Distribution Map of *Tigrosa helluo*.

1938, L.M. Barlett, AMNH, 1♀; Middlesex Co., Burlington (42.51°N, 71.19°W), 9 May 1962, A.R. Brady, MCZ, 1♂, Cambridge (42.37°N, 71.11°W), no date, J.H. Emerton, MCZ, 1♀. *Connecticut*, Fairfield Co., New Canaan (41.15°N, 73.49°W), April 1951, M. Statham, AMNH, 1♀, Norwalk (41.11°N, 73.41°W), 10 June 1933, W.J. Gertsch, AMNH, 1♀ with parasitized egg sac; Hartford Co., New Britain (41.68°N, 72.78°W), 8 June 1960, 1♀, 6 July 1961, A.J. Nappi, AMNH, 1♀ with young, West Hartford (41.76°N, 72.74°W), 28 May 1979, Sorkin & Klemens, AMNH, 1♀, Windsor (42.51°N, 73.06°W), 8 June 1960, A.J. Nappi, AMNH, 1♀ with young; New London Co., Colchester (41.57°N, 72.33°W), 11 May 1938, B.J. Kaston, AMNH, 1♂; New Haven Co., Guilford (41.28°N, 72.68°W), February 1937, J.M. Whiteside, AMNH, 1♀, Hamden (41.38°N, 72.90°W), 15 December 1936, W. MacFarland, AMNH, 1♀, Hamden, Mt. Carmel (41.42°N, 72.91°W), 26 August 1936, K. Sommerman, AMNH, 1♀, Hamden, Whitneyville (41.34°N, 72.91°W), 9 August 1932, B.J. Kaston, 1♀, New Haven, Westville (41.28°N, 72.68°W), 5 June 1933, B.J. Kaston, AMNH, 1♂, 1♀; Tolland Co., Storrs (41.82°N, 72.25°W), May 1935, G. Tolles, AMNH, 1♀, 25 May 1935, G. Nettleton, AMNH 1♀; *New York*, Albany Co., Rensselaerville (42.52°N, 74.14°W), 1 August 1948, F.S.E. & S.T. Green, AMNH, 1♀ with young, 17 August 1948, S.C. Bishop, AMNH, 1♀ with egg sac, 18 August 1948, S.C. Bishop, AMNH, 1♀, 19 September 1949, F. Harper, AMNH, 1♀ with egg sac; Clinton Co., Beekmantown (44.77°N, 73.50°W), 2 September 1962, E. Davis, AMNH, 1♀; Kings Co., Bergen Beach (40.62°N, 73.91°W), 29 April 1906, no name, AMNH, 2♀, 6 May 1906, no name, AMNH, 2♀, 30 May 1907, no name, AMNH, 2♀ with 1 egg sac, no name, 4 July 1907, AMNH, 3♀ with 2 egg cases, 23 May 1908, L.F. Barmann, AMNH, 3♀, 16 May 1909, O. Chiles, AMNH, 2♂, 2♀ with egg sacs, 12 May 1911, O. Chiles, AMNH, 1♀, Brooklyn (40.69°N, 73.99°W), September 1940, W. Spector, AMNH, 1♀; Madison Co.,

Hamilton (42.83°N, 75.54°W), 6 May 1910, L.F. Barmann, AMNH, 1♀; Nassau Co., Long Beach (40.59°N, 73.67°W), 3 May 1955, W. Ivie, AMNH, 3♀; New York Metropolitan Area: Bronx Borough, Harlem (40.81°N, 73.95°W), 12 June 1907, no name, AMNH, 1♀, Brooklyn Borough, Forest Park (43.75°N, 78.48°W), 27 September 1908, no name, AMNH, 1♀, 19 May 1909, L.T. Barnum, AMNH, 1♀, Queens Borough, Flushing (40.74°N, 73.83°W), 14 July 1912, H. Boyle, AMNH, 1♀, April 1937, 1♀, 18 March 1937, S.C. Bishop, AMNH, 4♀, Flushing Meadow (40.76°N, 73.83°W), 23 June 1962, 1♂, 30 June 1962, R.W. Fredrickson, AMNH, 1♀, Jamaica, (40.70°N, 73.81°W), 23 May 1909, O. Chiles, AMNH, 2♀, 1 egg sac; Rockland Co., Sloatsburg (41.15°N, 74.19°W), 20 September 1934, W.J. Gertsch, AMNH, 1♀; Schuyler Co., Tyrone (42.42°N, 77.07°W), 1910, no name, AMNH, 1♂, 1♀, no date, R.V. Chamberlin, AMNH, 1♂, 3♀; Seneca Co., Waterloo (42.89°N, 76.87°W), 3 September 1977, G. Dingerkus, AMNH, 1♀; Suffolk Co., Cold Spring Harbor (40.87°♀N, 73.46°W), 25 June 1903, no name, AMNH, 1♀ with young, 6 August 1908, no name, AMNH, 1♂, 16 June 1932, 3♀, 19 June 1932, W.J. Gertsch, AMNH, 2♀ with 2 egg sacs, July–August 1950, E. Mayr, AMNH, 1950, 1♀ with egg sac, Greenport (42.24°N, 73.77°W), 1 May 1949, R. Latham, AMNH, 1♀, 2 mi. [3.2 km] SW of Greenport, W. Ivie, 8 May 1955, AMNH, 2♀, Hampton Bays (40.87°N, 72.52°W), 20 June 1934, no name, AMNH, 1♀, Long Island, (40.80°N, 72.62°W), no date, no name, AMNH, 1♀, 30 April 1891, N. Pike, AMNH, 3♀, 3i, Montauk (41.03°N, 71.95°W), 7 June 1931, ♀ with 1 egg sac, 5 July 1931, 1♀, 17 June 1932, R. Latham, AMNH, 1♀, Orient (41.14°N, 72.30°W), 30 September 1932, 1♀, 10 May 1934, 1♀, 3 June 1948, 1♀, 1952, R. Latham, AMNH, 1♀ with egg sac, Riverhead (40.94°N, 72.64°W), 3 October 1956, R. Latham, AMNH, 1♂; Tompkins Co., Danby (42.35°N, 76.48°W), 27 July 1975, G. Dingerkus, AMNH, 1♀, Dryden (42.49°N, 76.30°W), 9 September 1974, G. Dingerkus, AMNH, 1♀ with young, Ithaca (42.44°N, 76.50°W), May 1902, no name, AMNH, 1♀, June 1905, no name, AMNH, 2♀ with 1 egg sac, 4 September 1976, G. Dingerkus, AMNH, 1♀, McLean (42.55°N, 76.29°W), 28 September 1912, no name, AMNH, 1♀, 28 September 1918, no name, AMNH, 1♀; Ulster Co., Ashokan (41.98°N, 74.20°W), 31 July 1909, no name, AMNH, 4♀, Phoenica (42.08°N, 74.32°W), July 1906, L.T. Barnum, AMNH, 1♂, Saugerties (42.08°N, 73.95°W), 15–20 August 1911, no name, AMNH, 1♀, 1 September 1911, no name, AMNH, 3♂, 3♀; Warren Co., Lake George (43.43°N, 73.71°W), June 1937, S.C. Bishop, AMNH, 1♀; Winchester Co., Montrose (43.35°N, 78.48°W), summer 1952, R.E. Thurston, AMNH, 2♀. *New Jersey*, Bergen Co., Edgewater (40.83°N, 73.58°W), 31 May 1909, no name, AMNH, 2♀ with 4 egg sacs, 16 June 1911, B. von Kochous, AMNH, 1♀, Ramsey (41.06°N, 74.14°W), 24 September 1934, no name, AMNH, 2♂, 2♀, 11i, 31 May 1935, no name, AMNH, 2♀, 3 August 1935, W.J. Gertsch, AMNH, 1♂, 1–10 June 1944, W. J. Gertsch, AMNH, 1♀, Tenafly (40.92°N, 73.97°W), 12 September 1907, AJ. Nappi, AMNH, 1♀ with young; Cape May Co., Ocean City (40.93°N, 73.97°W), 6 June 1961, M.N. & G.P. Feinberg, AMNH, 1♀ with young; Essex Co., Montclair (40.82°N, 74.22°W), 20 June 1907, no name, AMNH, 2♀; Hunterdon Co., Lambertville (40.37°N, 74.95°W), September 1951, 2♀, June 1955, W. Ivie, AMNH, 2♀; Mercer Co., Pennington (40.33°N, 74.79°W), 26 September 1939, K.W. Cooper, AMNH, 1♀, Princeton (40.36°N, 74.66°W), 13 October 1939, K.W. Cooper, AMNH, 1♀, Trenton (40.22°N, 74.76°W), 6 August, no name, AMNH, 1♀. *Pennsylvania*, Berks Co., Shillington (40.30°N, 75.97°W), 20 June 1937, L. Hook, AMNH, 2♂, Virginville (40.52°N, 75.87°W), October 1965, P. Vaurie, AMNH, 1♀; Bradford Co., Wilawana (41.99°N, 76.60°W), June 1939, R. Crandall, AMNH, 2♀; Chester Co., Valley Forge (39.84°N, 75.47°W), 4 November 1966, AMNH, E. Knauss, 1♀; Fayette Co., Horseshoe Bend, Neshaminy Creek, NE of Jamison (40.26°N. 75.09°W), 17 May 1953, 1♂, 3♀, July 1953, 1♀, 21–31 August 1953, 6♂, 8 September 1953, 1♀, 25 September 1953, 1♀, March 1954, 1♀, April 1954, 4♀, May 1954, 1♂, 5♀ with 2 egg AMNH, W. Ivie, 1♀, 23 May 1965, J. & W. Ivie, AMNH, 1♂, 2♀, 1 egg sac; Lancaster Co. (40.05°N, 76.18°W), August 1887, Stone, AMNH, 2♀, Lancaster (40.05°, 76.18°), June 1954, 5♂, 3♀ with one egg sac, July 1954, 2♂, 3♀, May 1955, 2♂, 2♀, July 1955, 3♂, 1i, September 1956, 2♂, September 1957, AMNH, 5♂, 1♀, July 1964, W Ivie, AMNH, 1♀; Montgomery Co., North Wales (40.21°N, 75.28°W), 19 July–9 August 1944, V.M. von Hagen, AMNH, 1♂; Northampton Co., Easton (40.69°N, 75.21°W), August 1943, no name, 1♀, Wind Gap (40.84°N, 75.29°W), 8 August 1943, no name, 1♀ with egg sac, Philadelphia Co., Philadelphia (39.95°N, 75.18°W), May 1909, L.T. Barnum, AMNH, 3♀; Wabash Co., (40.99°N, 77.60°W), 17 March 1930, W.W. Long, AMNH, 2♀; Washington Co., (40. 21°N, 80.18°W), 17 March 1930, W.W. Long, AMNH, 2♀, 4i; York Co., 4 mi. [6.4 km] N of Waterford (39.97°N, 76.69°W), 12 August 1962, D. Kurczewski, AMNH, 1♀. *Ohio*, Hocking Co., Cantwell Cliffs near Rockbridge (39.56°N, 82.60°W), 27 July 1935, W. Ivie, AMNH, 1♀; Knox Co., (40.41°N, 82.46°W), no name, AMNH, 1♀; Perry Co., New Lexington (39.71°N, 82.21°W), 22 July 1873, Holden, AMNH, 1♂; Preble Co., 2 mi. [3.2 km] S of New Paris (39.86°N, 84.79°W), 26 August 1950, V. Roth, AMNH, 1♂. *Maryland*, Anne Arundel Co., Patuxent (39.05°N, 76.74°W), May 1942, no name, AMNH, 1♂, 2♀, 4i; Cecil Co., Elk Neck (39.51°N, 75.95°W), 17 May 1964, J. & W. Ivie, AMNH, 1♀; Dorchester Co., Cambridge (38.56°N, 76.08°W), 28 May 1979, G. Price, HCC, 1♀; Montgomery Co., Bethesda (38.98°N, 77.09°W), 25 April 1944, 1♀, 18 October 1944, J.M. Davis, AMNH, 1♂, Kensington (39.03°N, 77.08°W), 21 August 1945, J.M. Davis, AMNH, 1♀ with young. *District of Columbia*, Washington D.C. (38.89°N, 77.03°W), 1 June 1944, J.M. Davis, AMNH, 1♀, 4 October 1945, B. Malkin, AMNH, 1♂. *West Virginia*, Mercer Co., Princeton (34.99°N, 81.10°W), 28 August 1968, N.I. Platnick, AMNH, 1♀; *Ohio Co.*, Wheeling (40.10°N, 80.63°W), August–October 1947, K.W. Haller, AMNH, 1♀; *Pocahontas Co.*, Minnehaha Springs (38.16°N, 79.98°W), July 1947, K.W. Haller, AMNH, 1♂, 2♀. *Virginia*, Alleghany Co., Clifton Forge (37.81°N, 79.82°W), April 1950, R.L. Hoffman, AMNH, 2♂, 1♀; Augusta Co., Mint Spring, S of Staunton (38.15°N, 79.07°W), no date, no name, AMNH, 1♀; Bath Co., (38.05°N, 79.75°W), no date, no name, MCZ, 1♂, 1♀; Page Co., Luray (38.67°N, 78.46°W), 1–7 October 1943, AMNH, 1♀, 8–17 April 1946, AMNH, B. Malkin, 1♀; Portsmouth (Independent City) (36.83°N, 76.30°W), 5–13 May 1968, E. Sabath, AMNH, 1♂. *Kentucky*, Boyle Co., 3 mi. [4.8 km] SE of Danville (37.65°N, 84.77°W), 29 June–4 July, S.C. Bishop, AMNH, 1♀;

Christian Co., Hopkinsville (36.87°N, 87.49°W), 10 March 1929, no name, AMNH, 1♂; Fulton Co., (36.50°N, 88.87°W), no date, no name, AMNH, 1♀; Hardin Co., Summit (37.58°N, 86.04°W), 1 May 1944, B. Malkin, AMNH, 2♀, 1i; Jasmine Co., Valley View (37.85°N, 84.43°W), 28 June 1925, no name, AMNH, 1♀. *Tennessee*, Cheatam Co., Kingston Spring (36.10°N, 87.11°W), 10–15 July 1933, W.J. Gertsch, AMNH, 1♂, 1♀, 2i; Davidson Co., Nashville (36.17°N, 86.78°W), 27 April 1957, no name, AMNH, 1♂; Sevier Co., Greenbriar Cove, Great Smoky Mountains National Park (35.60°N, 83.50°W), 3 June 1939, A.E. Cole, AMNH, 1♀; Wayne Co., Factory (35.52°N, 86.58°W), 4 May 1976, G. Dingerkus & R.A. Stiles, AMNH, 1♀; Wilson Co., Cedars of Lebanon State Park (36.09°N, 86.34°W), 10 May, A.R. Brady, HCC, 3♀. *North Carolina*, Lee Co., Deep River near Sanford (35.47°N, 79.16°W), 25 April 1938, W.J. Gertsch, AMNH, 8♀ with 1 egg case; Moore Co., Manly (35.19°N, 79.37°W), October 1955, A. Twombly, AMNH, 1♂, 1♀, 2i; Swain Co., Nantahala (35.34°N, 83.62°W), summer 1952, H.I. Gillies, AMNH, 1♀; Union Co., Monroe (34.98°N, 80.55°W), June 1942, Mrs. E L. Bell Jr., AMNH, 1♂. *South Carolina*, Chesterfield Co., Cheraw (34.70°N, 79.88°W), 22 August 1933, no name, AMNH, 3♀; McCormick Co., 4 mi. [6.4 km] N of Modoc (33.74°N, 82.21°W), 29 May 1964, A.R. Brady, HCC, 1♀, 1i. *Georgia*, Clarke Co., Athens (33.96°N, 83.37°W), 7 August 1929, Richards, AMNH, 1♂, 19 June 1953, H.O. Lund, AMNH, 1♀ with egg sac; Charleston Co., Folkston (30.83°N, 92.01°W), 18 February 1938, no name, AMNH, 1♀; Hall Co., Gainesville (34.30°N, 83.83°W), 24 April 1943, W. Ivie, AMNH, 3♀; Screven Co., Sylvania (32.76°N, 81.65°W), 9 April 1943, 1♀, N of Sylvania, 15 April 1943, 1♀, 17 April 1943, W. Ivie, AMNH, 1♀, 1i. *Florida*, Alachua Co., Paynes Prairie, Gainesville (29.65°N, 82.32°W), 10 March 1936, no name, AMNH, 2♀, 1i; Bay Co., Panama City (30.16°N, 85.66°W), 29 March 1965, A.R. Brady, HCC, 1♂; Collier Co., 17.2 mi. [27.68 km] N of Jerome (27.47°N, 81.52°W), 6 April 1952, A. Schwartz, AMNH, 2♀, 18.2 mi. [29.29 km] W of Monroe Station (27.47°N, 81.52°W), 18 April 1952, A. Schwartz, AMNH, 1♂, 1♀; Dade Co., Florida City (35.45°N, 80.48°W), 31 March 1957, W.J. Gertsch, R. Forster, AMNH, 1♀, 20 mi. [32.2 km] W of Miami (25.73°N, 80.24°W), 31 April 1952, A. Schwartz, AMNH, 1♀, Big Pine Key (34.67°N, 81.35°W), 16 June 1962, A.R. Brady, HCC, 1♀; Hernando Co., Aripeka (28.43°N, 82.67°W), 2 June 1964, J. Reiskind, HCC, 1♀ with egg sac; Highlands Co., Highlands Hammock State Park (27.47°N, 81.52°W), 20 April 1973, N.R. Spencer, HCC, 1♂, 1♀, 1 June 1973, A.R. Brady, HCC, 3♀; Lee Co., Fort Myers (26.64°N, 81.87°W), no date, W.J. Gertsch, AMNH, 1♀; Levy Co., Manatee Springs State Park (29.50°N, 82.97°W), 11 June 1961, 2♂, 21 June 1962, A.R. Brady, HCC, 1i; Liberty Co., Torreya State Park (30.55°N, 84.95°W), 1 June 1964, A.R. Brady, HCC 1♂, 1♀, 18 May 1973, A. Jung, HCC, 2♂, 1♀; Martin Co., Port Mayaca (26.99°N, 80.61°W), 29 March 1938, W.J. Gertsch, AMNH, 1♂, 2♀ with 1 egg sac; Orange Co., Winter Park (28.59°N, 81.35°W), 21 March 1938, W.J. Gertsch, AMNH, 1♀; Pasco Co., 10 mi. [16.1 km] S of Zephyrhills (28.23°N, 82.18°W), 7 April 1938, W.J. Gertsch, AMNH, 3♂. *Alabama*, Greene Co., Eutaw (32.84°N, 87.89°W), 9 July 1950, M. Cazier, AMNH, 2♀; Jackson Co., (34.73°N, 85.97°W), 23 July 1910, no name, AMNH, 1♀. *Mississippi*, Adams Co., Selma (32.41°N, 87.02°W), R.V. Chamberlin, 25 July 1910, AMNH, 1♀; Pike Co., Fernwood (31.19°N, 90.45°W), 19 July 1910, R.V. Chamberlin, AMNH, 1♂, 2♀; Pontotoc Co., 1 mi. [1.6 km] SE of Ecru (34.35°N, 89.03°W), no date, W.H. Cross, MSST, 1♂; Rankin Co., Thompson Field (32.32°N, 89.99°W), 10 September 1983, T.C. Lockley, HCC, 1♀; Washington Co., (33.27°N, 90.96°W), 5 June 1987, 1♀, 8 June 1987, T.C. Lockely, HCC, 1♀ with young, Stoneville (33.42°N, 90.92°W), 18 April 1982, T.C. Lockley, HCC, 1♀. *Louisiana*, Caddo Par., Shreveport (32.51°N, 93.75°W), 10 April 1948, J.H. Robinson, AMNH, 1♀; Cameron Par., 1 mi. [1.6 km] N of Johnson Bayou by State Highway 82 (33.80°N, 82.27°W), 23 March 1974, R.L. Ervin, AMNH, 1♀ with egg sac; Madison Par., Tallulah (32.41°N, 91.19°W), no date, no name, AMNH, 1♀; St. Charles Par. (29.89°N, 90.36°W), Bonnet Carre Spillway, 10 February 1950, E.N. Lambarement, AMNH, 1♀. *Michigan*, Allegan Co., Hope College Field Station (42.59°N, 85.91W), 18 September 1978, A.R. Brady, HCC, 1♀, 4 mi. [6.4 km] S of New Richmond (42.59°N, 86.11°W), 17 July 1966, A.R. Brady, HCC, 1♀, Holland (42.77°N, 86.10°W), 12 June 1975, B. Ross, HCC, 1♀ with egg sac, Zoerman Farm, Holland (42.77°N, 86.10°W), 22 June 1995, N.T. Harmon, HCC, 1♀, Mann Creek, Holland (42.77°N, 86.10°W), 12 May 1971, K. Ringsmith, HCC, 1♀; Barry Co., Otis Lake (42.61°N, 85.42°W), 24 June 1960, N.I. Platnick, AMNH, 1♀; Calhoun Co., Albion (42.25°N, 94.75°W), 27 June 1930, 1♀, 3 June 1935, 1♀, 2 June 1949, 1♀, A.M. Chickering, MCZ, 1♀; Charlevoix Co. (45.25°N, 85.06°W), 27 July 1938, A.M. Chickering, MCZ, 1♂, 4 mi W of Petoskey (45.37°N, 84.96°W), 29 July 1937, A.M. Chickering, MCZ 1♂; Cheboygan Co., (45.65°N, 84.48°W),17 June 1930, no name, MCZ, 1♂, July 1944, no name, MCZ, 1♀ with egg sac, Bois Blanc Island (45.77°N, 84.47°W), 2 August 1932, A.M. Chickering, MCZ, 3♀, with 1 egg sac, Douglas Lake (45.53°N, 84.92°W), 10 August 1931, 1♂, 12 August 1931, 1♂, 9 August 1938, 1♀, A.M. Chickering, MCZ, 1♀; Emmet Co., Bayview (45.39°N, 84.93°W), 21 July 1937, 1♀ with egg sac, 14 July 1938, 1♀ with egg sac, July 1941, A.M. Chickering, MCZ, 1♀, Petoskey (45.37°N, 84.96°W), 29 July 1937, 1♂, 18 July 1940, A.M. Chickering, MCZ, 1♀ with egg sac; Kalkaska Co., 12 mi. [19.3 km] W of Kalkaska (44.74°N, 85.17°W), 28 June 1975, B. Witzel, HCC, 1i; Kent Co., Chain Lakes (43.18°N, 85.33°W), 7 June 1975, B. Witzel, HCC, 1♂; Lenawee Co., 3 mi. [4.8 km] SW of Jasper (41.79°N, 84.04°W), 10 October 1975, P. Schuch, HCC, 1♂, 2♀, 1i; Livingston Co., E.S. George Reserve (42.47°N, 84.00°W), 4 October 1936, 1♀, 7 August 1939, I.J. Cantrall, FSCA, 1♂, 21♀, 3i, 28 July 1951, H.K. Wallace, FSCA, 1♀, 10 May 1953, E.N. Pruitt, FSCA, 1♀; *Midland Co.*, (43.65°N, 84.39°W), July 1931, 1♀, 26 August 1933, 1♀ with egg sac, 22 June 1942, A.M. Chickering, MCZ, 1♀, 14 July 1948, R.R. Dreisbach, MCZ, 1♀; Muskegon Co., Muskegon (42.23°N, 86.25°W), 10 August 1945, R.R. Dreisbach, MCZ, 1♀; Ottawa Co., (42.99°N, 86.03°W), S. Schnienzer, HCC, 10 April 1984, Grand Haven (43.06°N, 86.23°W), 20 September 1933, H. Rearwin, AMNH, 1♂, Holland (42.77°N, 86.10°W), 30 September 1968, W. Defeyter, HCC, 1♀, May 1969, D. Michael, HCC, 1♂; Shiawassee Co. (42.95°N, 84.15°W), Rose Lake Experimental Station, 28 May 1958, 1♂, 1♀, 23 May 1966, 2♀, 28 May 1966, E. Evans, AMNH, 3♀; Washtenaw Co., (42.25°N, 83.84°W),

Waterloo Recreation Area, 31 May 1941, A.M. Chickering, MCZ, 2♂, 1♀; 15–31 August, A. Peacock, MCZ,1♀. *Indiana*, Kosciusko Co., Webster Lake (41.21°N, 86.11°W), 14 May 1946, no name, AMNH, 1♀; Monroe Co., Bloomington (39.17°N, 86.53°W), 22 July 1949, L. Griffey, AMNH, 1♀ with egg sac; Owen Co., Spencer (39.29°N, 86.76°W), Spring 1976, J. Lyons, AMNH, 1♀; Parke Co., Turkey Run State Park (39.88°N, 87.21°W), 20 May 1932, F. Clarke, AMNH, 4♀, 1i; Porter Co., Chesterton (41.61°N, 87.06°W), 24 June 1939, D.C. Lowrie, AMNH, 1♀, 2i. *Wisconsin*, Crawford Co., Prairie du Chien (43.05°N, 91.14°W), September 1944, L. Smethuis, AMNH, 2♂, 6 May 1953, M. Melanie, AMNH, 1i; Marinette Co., Marinette (45.10°N, 87.63°W), 5 July 1910, AMNH, R.V. Chamberlin, AMNH, 2♀, 1 egg sac; Rock Co., Beloit (42.51°N, 89.04°W), 7 July 1910, R.V. Chamberlin, AMNH, 1♀; Walworth Co., Elkhorn (42.67°N, 88.54°W), 12 July 1938, F. Farn, AMNH, 1♀. *Illinois*, Cook Co., Chicago (41.88°N, 87.63°W), 10 July 1932, W.J. Gertsch, AMNH, 1♀; Henderson Co., Biggsville (40.85°N, 90.86°W), 20 July 1935, W. Ivie, AMNH, 1♀; McLean Co., Bloomington (40.49°N, 89.00°W), no date, H.W. Britcher, AMNH, 1♀, 1i; Peoria Co., Peoria (38.21°N, 89.97°W), 8 July 1910, 1♂, 1 September 1910, R.V. Chamberlin, AMNH, 4♀ with 2 egg sacs; Tazewell Co., East Peoria (40.79°N, 89.60°W), 10 July 1910, R.V. Chamberlin, AMNH, 1♀ with young. *Minnesota*, Lake Co., Wacouta Beach, Lake Pepin (47.57°N, 91.41°W), 15 May 1932, W.J. Gertsch, AMNH, 1♀. *Iowa*, Boone Co., Boone (42.06°N, 93.90°W), 23 June 1910, R.V. Chamberlin, AMNH, 4♀, Ledges State Park (42.06°N, 93.90°W), 19 May 1941, D.T. Jones, AMNH, 3♀, Mongona (42.02°N, 93.93°W), 22 June 1910, R.V. Chamberlin, AMNH, 3♀; Clinton Co., DeWitt (41.82°N, 90.54°W), 26 June 1910, R.V. Chamberlin, AMNH, 1♀, 1i; Story Co., Ames (42.02°N, 93.63°W), Spring 1941, D.T. Jones, AMNH, 1♀; *Missouri*, St. Charles Co., St. Charles (38.78°N, 90.48°W), 1927, M.B. Brown, AMNH, 1♀; St. Louis Co., Ranken (38.53°N, 90.51°W), 29 July 1945, E. P. Meiners, AMNH, 2♀, 4i; Vernon Co., Nevada (37.84°N, 94.36°W), 30 May 1960, D. Lamore, MCZ, 1♀. *Nebraska*, Cass Co., Plattsmouth (41.01°N, 95.90°W), 28 March 1923, W. Ivie, AMNH, 1i; Richardson Co., 10 mi. [16.1 km] N of Falls City (40.06°N, 95.60°W), 7 June 1933, W. Ivie, AMNH, 2i. *Kansas*, Wyandotte Co., Kansas City (39.11°N, 94.63°W), 8 June 1933, W. Ivie, AMNH, 2♀. *Texas*, Harris Co., Houston (29.76°N, 95.37°W), November 1935, S. Mulaik, AMNH, 1♀. *California*, Placeer Co., Auburn (38.90°N, 121.08°W), 11 May 1951, E.I. Schlinger, AMNH, 1♂, not mapped.

Diagnosis.—*Tigrosa helluo* can be distinguished from *T. aspersa* by its smaller size. The average body length of *T. helluo* is 17 mm, whereas in *T. aspersa* the average is 28 mm. Compare Table 5 with Table 2 for additional size differences. The distinct longitudinal stripe on the carapace that begins in the AME region and continues to the posterior declivity in *T. helluo* females (Fig. 28) is represented in *T. aspersa* females (Fig. 10) by a shortened stripe or dash in the eye region. *Tigrosa helluo* (Fig. 28) does not display the short distinct pale dashes behind the AME on each side of the median stripe and the vivid broad cream colored markings surrounding the dark lanceolate cardiac marks, nor does it display the lighter markings of the posterior region that occur in *T. annexa* (Figs. 1–3). *Tigrosa helluo* and *T. georgicola* have very similar dorsal patterns on the cephalothorax and abdomen; however, the pale submarginal stripes in *T. helluo* (Figs. 28, 29) are narrower and do not extend to the carapace margin as in *T. georgicola* (Figs. 16, 17). In addition the venter in *T. helluo* is marked by small black spots (Fig. 41), while *T. georgicola* has several rows of spots or dashes, often coalescing into stripes that converge in front of the spinnerets (Fig. 42), and the venter is occasionally entirely black. The LP of the epigynum in *T. helluo* (Fig. 33) widens slightly anteriorly almost to the anterior margin of the epigynum, while in *T. georgicola* (Fig. 18) it flares outward a considerable distance before the anterior margin and is much wider. The median apophysis (MA) in *T. helluo* (Fig. 30) is less developed than in *T. georgicola* (Fig. 20). The palea in *T. helluo* (Fig. 31) is rounder than in *T. georgicola* (Fig. 21) where it tends to be quadrangular, and the sclerotized ridges on the palea of *T. helluo* are less developed than in *T. georgicola.*

Color.—*Female:* Dorsal pattern illustrated in Fig. 28. Face reddish brown. Chelicerae dark reddish brown with condyles dark red. Eye nacelles black. Carapace reddish brown with black lines radiating from thoracic groove to submarginal stripes. Carapace with thin yellow stripe originating between AME, expanding slightly posterior to PME row, and continuing to posterior declivity; light brown to yellow-brown submarginal stripes with scallops or indentations along edges. Dorsum of abdomen brown; lanceolate cardiac mark lighter brown with dark brown to black margins; often with small lighter dots lateral to cardiac region. Venter of abdomen pale brownish yellow to cream in the middle region and characterized by dark brown or black spots, with darker brown lateral to central area (Fig. 41). Legs reddish brown without distinct bands, although dusky markings on femora occur in some specimens; ventral surfaces of tibiae and femora lighter yellowish brown. Labium and endites dark reddish brown to black. Distal ends of labium and endites lighter yellowish. Sternum and coxae dark reddish brown to black.

Male: Dorsal pattern illustrated in Fig. 29. Face brownish yellow; lateral regions darker brownish. Dark spots under ALE. Chelicerae brownish yellow with condyles darker brown. Eye region of carapace dark brown; eyes circled in black. Carapace mostly brown with darker lines radiating from thoracic groove to submarginal stripes. Narrow yellow median stripe from PME to posterior declivity. Stripe thinnest between PLE and widening as it approaches and surrounds black thoraeic groove. Narrow light brown or yellow uneven submarginal stripes. Dorsum of abdomen brown with conspicuous lanceolate cardiac mark that is lighter brown, enclosed with black and bounded by lighter yellow. Four or five faint darker chevrons extending posteriorly from cardiac area. Venter of abdomen yellow or cream with numerous dark brown spots. Legs yellow without darker markings, lighter on ventral surfaces. Labium yellow, brownish at proximal end. Endites yellow without darker color. Sternum yellow with darker brown perimeter and two dark central stripes, widening posteriorly.

Natural history.—Kaston (1948) reported *T. helluo* from woods, salt marshes and grassy areas in Connecticut. In Michigan I have found this species most often near the edge of lakes and in marshy areas. In Florida and Mississippi *T. helluo* also seems to prefer similar wetter areas unlike *T. georgicola,* which is more often found in drier wooded areas. According to Kaston (1948) mature males occur from May to September in

Connecticut and females throughout the year. Apparently females overwinter in the adult stage. Mating occurs in June, and the mating behavior has been recorded by Kaston (1936).

Distribution.—Recorded from Ontario, Canada. In USA *Tigrosa helluo* is found from New England in the northeast, south along the eastern seaboard to Florida, then north and eastward throughout much of the United States to the one-hundredth meridian (Map 5).

Hogna Simon, 1885

Biarabenia Mello-Leitao 1941:137 (part).
Lycorma Simon 1885:9 (part).
Isohogna Roewer 1960:569 (part).
Lynxosa Roewer 1960: 901 (part).
Citilycosa Roewer 1960:846 (part).

Diagnosis.—*Hogna* is distinguished from other large lycosids by the dorsal color pattern on the carapace that consists of a broad cream to yellow median stripe that begins in the facial or frontal area and then extends posteriorly from the facial area to the posterior declivity of the cephalothorax. The width of the median stripe is equal to or exceeds the distance between the PME. In addition, broad scalloped submarginal stripes extend from the cephalic region of the carapace to the posterior declivity. The submarginal stripes sometimes reach to the margins of the carapace, particularly in males. A darker color on the carapace that ranges from medium to dark brown provides a contrasting darker background (Figs. 34, 35). The dark brown or black cardiac mark on the dorsum of the abdomen in *Hogna* is often outlined in a lighter color that is accented by two distinct black dots along the posterior margin (Figs. 34, 35). The venter of the abdomen is largely black, with the color extending from the anterior margin to the base of the spinnerets (Fig. 45). The AER width in *Hogna* is less than the width of the PME row (0.30 mm or more difference), and the POQ length is greater than the width of the AER (0.14 mm or more difference). In the epigynum of *Hogna* the LP is much longer than the TP (Fig. 39), the sides are parallel and there are lateral grooves along the length of the LP (Fig. 39). The structures of the male palpal organ (e.g., embolus, median apophysis, tegulum and tegular apophyses) are plesiomorphic and do not exhibit enough differences to separate *Hogna* from other lycosine genera.

Remarks.—The above preliminary diagnosis is derived from specimens identified as *Hogna* in collections that I have examined. It is based primarily upon collections from the Natural History Museum, London, and the Muséum national d'Histoire naturelle in Paris, as well as additional specimens from France and Italy sent by individual collectors. The focal point of this diagnosis is *Hogna radiata*, the type species of the genus, but it also includes a number of closely related species that occur in northern Africa. Although several of these North African specimens are labeled as *Hogna radiata,* they are quite distinct. Until the geographic range of *H. radiata* itself is determined, I thought it best to offer a conservative diagnosis for *Hogna.* It is very possible that the genus *Hogna,* like the genus *Lycosa,* is largely restricted to the Mediterranean region and is not found in North America.

Simon (1885) listed *Hogna* as a Group under the Genus *Lycosa.* Simon (1898) briefly described *Hogna* and he assigned it to *Lycosa* under the Subgenus *Hogna* (Section IE) with the type *Lycosa radiata.* Section I included all those species of *Lycosa* with three teeth on the posterior margin of the cheliceral groove. The technique used by Simon (1898) was then to compare *Hogna radiata* to the four preceding species of *Lycosa* designated as A–D under Section I. The following information is extracted from that description: AER procurved (less so than in the preceding species), the PME row not as wide as the PLE row; legs robust, with metatarsus IV and patella-tibia IV shorter than in the preceding species; clypeus, tarsi and the genital plate scopulate.

Roewer (1959) cites the first proposal of the name *Hogna* by Simon (1885) and then briefly discusses the diagnosis by Simon (1898). He notes that the genus *Hogna* was regarded by many authors after Simon (1898) as a genus separate from *Lycosa.* He does not think that the arrangement of the scopulae on the legs, to which Simon gives particular attention, is of much significance at the generic level. Roewer (1959) limits his diagnosis of *Hogna* to the following characteristics and emphasizes the arrangement and dimensions of the eyes and eye rows as especially significant. The following is a synopsis of the diagnosis by Roewer (1959): Labium longer than wide. Order of the length of legs IV-I-II-III. Metatarsus IV shorter than Patella-Tibia IV. Tibia I with no more than three pairs of ventral spines or macrosetae. Chelicerae with three teeth on the posterior margins of the cheliceral grooves. Eyes: Anterior row procurved, First row of eyes narrower than the second row. Distance between ALE and AME is equal to distance between AME. AME larger than ALE. Distance between PME is less than 1 diameter of PME. After the emphasis on eye size and arrangement in the diagnosis of *Hogna,* Roewer (1959) uses these features to defend the transfer of eight species from *Hogna*, to *Allocosa, Dingosa, Trochosa* and *Artoriellula* and to transfer six species from *Trochosa, Geolycosa, and Schizocosa* to *Hogna.* Finally he questions the placement of over twenty species in the genus *Hogna* because they have not been sufficiently diagnosed by the authors. This brief view of nomenclatural history is to emphasize the difficulty produced by the promulgation of Roewer (1959) of an artificial system of classification, the carte blanche application of the size and position of eyes. This system (Roewer 1959) lacks value in discriminating the many genera that comprise the family Lycosidae. It has placed a large roadblock in the path to understanding systematic relationships of lycosid genera. According to the rules of nomenclature, however, the names and changes introduced by Roewer must be individually evaluated for their validity. In my own diagnoses of *Hogna* and *Tigrosa* I have tried to incorporate a larger number of characteristics that may shed light on phylogenetic relationships, including dorsal color pattern of cephalothorax and abdomen, eye arrangement, dimensions of body and legs, and most importantly the details of male and female genitalic characters.

Hogna radiata (Latreille 1817)

Figs. 34–39, 45, Table 6

Lycosa radiata Latreille 1817:292; Simon 1876:60, 87, 1937:1094, 1132; Guy 1966:85; Fuhn & Oltean 1969:167; Fuhn & Niculescu-Burlacu 1971:195; Miller 1971:154; Loksa 1972:49; Mcheidze 1997:227.

Hogna radiata Roewer 1955:249, 1959:403; Zyuzin 1993:699; Thaler, Buchar & Knoflach 2000:1076; Deltshev & Blagoev 2001:110; Deltshev 2003:137; Trotta 2005:169.

Table 6.—Mean and range of ten males and ten females of *Hogna radiata* from Western Europe.

	Mean (range)		Mean (range)
Females			
Anterior eye row	1.56 (1.4–1.7)	Femur I	6.89 (6.0–7.7)
PME width	1.89 (1.7–2.0)	Patella-Tibia I	8.83 (8.0–10.1)
PLE width	2.39 (2.2–2.6)	Metatarsus I	5.29 (4.8–6.0)
POQ length	1.70 (1.5–1.9)	Tarsus I	2.99 (2.7–3.3)
Car. width at PLE	4.18 (3.7–4.7)	Total length I	24.01 (21.5–27.0)
Carapace width	6.96 (6.4–7.7)	Femur IV	7.83 (7.0–8.8)
Carapace length	9.26 (8.1–10.0)	Patella-Tibia IV	9.34 (8.6–10.4)
Body length	19.83 (17.4–22.5)	Metatarsus IV	8.70 (7.8–9.8)
Patella-Tibia II*	8.07 (7.4–9.4)	Tarsus IV	3.46 (2.9–3.7)
Patella-Tibia III	6.98 (6.4–7.8)	Total length IV	29.33 (26.9–32.6)
Males			
Anterior eye row	1.30 (1.2–1.6)	Femur I	6.85 (5.9–8.1)
PME width	1.51 (1.3–1.7)	Patella-Tibia I	8.98 (7.7–10.8)
PLE width	1.94 (1.7–2.2)	Metatarsus I	6.05 (5.3–7.6)
POQ length	1.38 (1.2–1.7)	Tarsus I	3.44 (2.9–4.0)
Car. width at PLE	3.18 (2.7–3.9)	Total length I	25.32 (21.9–30.3)
Carapace width	5.85 (5.1–7.4)	Femur IV*	7.51 (6.7–9.0)
Carapace length	7.87 (6.5–9.6)	Patella-Tibia IV*	9.10 (8.1–10.9)
Body length	15.48 (13.4–19.0)	Metatarsus IV*	9.09 (8.1–11.3)
Patella-Tibia II	8.34 (6.7–10.0)*	Tarsus IV*	3.89 (3.3–4.7)
Patella-Tibia III	6.96 (5.3–8.4)*	Total Length IV*	29.59 (26.6–35.9)

* indicates N=9.

Remarks.—The recognition of the species described here as *Hogna radiata* is based upon examination of specimens from key geographic localities. The thorough diagnosis and excellent illustrations of *Lycosa radiata* by Fuhn & Niculescu-Burlacu (1971) were key elements in defining *Hogna radiata.* In addition to references to *Lycosa radiata* and *Hogna radiata* listed above, there are 14 different specific names for *H. radiata* listed as synonyms in the World Catalogue of Spiders (Platnick 2011) . Because of the lack of clarity concerning the true geographic range of *H. radiata* and the absence of a definitive definition of this species throughout its range, I chose to present above only a brief list of selected references for this species. For details of this problem see Fuhn & Niculescu-Burlacu (1971). A complete list of systematic references may be found in Platnick (2011). The application of the name *Hogna radiata* to widespread and diverse populations brings into serious question the systematics of this species. Upon further study I think that several of the synonyms listed by Platnick (2011) will undoubtedly be resurrected as distinct species. The various subspecies names applied to different populations of *H. radiata* are also problematical.

Type material.—*Holotype:* Unable to locate.

Other material examined.—GERMANY: Freiburg (48.00°N, 07.85°E), 18 September 1919, no name, BMNH, 2♂. FRANCE: Montpellier (43.61°N, 3.88°E), 7 July 1969, C.D. Dondale, CNC, 1♂, 24 July 1969, C.D. Dondale, CNC, 1♀; 7 July 1969 Cerbère (42.44°N, 3.17°E), D.J. Clark, 25 June 1962, BMNH, 2♂, 5♀. SPAIN: Laviana (45.25°N, 5.56°W), no date, no name, MNHP, 1♂, 1♀; Island of Menorca (= Minorca) (39.95°N, 4.06°E), D. Braun, BMNH, 1♂, 5♀.

Diagnosis.—An accurate or true diagnosis of *H. radiata* throughout its range is very difficult until the systematic relationships of the populations described under *H. radiata* are determined. The species name *H. radiata* has been applied to populations spread over broad geographic regions. Because a number of these populations are quite distinct, some of the names now listed as synonyms may need to be resurrected as valid species. The color pattern and measurements recorded below are taken from specimens from Western Europe, the area from which *H. radiata* was originally described. Comparisons between *H. radiata* and *Tigrosa* are based primarily upon these specimens. A brief comparison of *H. radiata* and *Tigrosa* is made earlier in the Remarks section under *Tigrosa*, new genus.

The diagnosis of *H. radiata* presented here is based primarily upon the examination of collections from the Natural History Museum, London, and the Muséum national d'Histoire naturelle in Paris, as well as additional specimens from France and Italy examined earlier. Based upon examination of these specimens and a review of the literature, it appears that the geographic range and taxonomic relationships of dissimilar populations of *H. radiata* are not clearly understood. It is not the intention of this paper to determine the range or relationships of these distinct populations described as *H. radiata.* Instead the diagnosis is focused upon the significant systematic differences between *H. radiata,* as portrayed by the specimens examined, and the new genus *Tigrosa.* In *Tigrosa* the presumed synapomorphy that connects its members is the dorsal pattern on the cephalothorax. It is characterized by a narrow cream to yellow median stripe on the carapace that begins in the AME region and continues to the posterior declivity. This stripe widens in the thoracic area, but its width throughout its length does not exceed the space between the PME (Figs. 28, 29). In *H. radiata* the pale yellow median stripe is much wider, and its width exceeds the space between the PME (Fig. 34, 35). In *T. lelluo* there are narrow yellow to light brown submarginal stripes beginning behind the PME row and continuing posteriorly (Figs. 28, 29). In *H. radiata* the pale submarginal stripes begin in the vertical facial area, are broader, and often reach to the margins of the carapace

(Figs. 34, 35). The cardiac mark on the dorsum of the abdomen in *T. helluo* is often outlined in dark brown or black (Figs. 28, 29). In *H. radiata* (Figs. 34, 35) the cardiac mark is also outlined in black but includes two distinct black dots along the posterior margin, a condition not found in *Tigrosa.* The ventral surface of the abdomen posterior to the epigastric furrow in *H. radiata* is entirely black (Fig. 45). In *Tigrosa* the venter of the abdomen is usually cream to light brown in overall color with scattered black spots (Figs. 40, 41), without conspicuous black dots or dashes in the central area (Fig. 43), or with spots or dashes arranged in longitudinal rows (Figs. 42, 44). Fundamental differences also occur between *Hogna* and *Tigrosa* in the eye arrangement. For example, the anterior eye row width in *Tigrosa* is subequal to the PME row width (0.17 mm or less difference), but in *Hogna radiata* the anterior eye row width is obviously less than the width of the PME row (0.30 mm. or more differenee). Also the length of the POQ in *Tigrosa* (with the exception of *T. aspersa*) is equal to the width of the anterior eye row (0.02 mm or less difference), but in *Hogna radiata* the POQ length is greater than the width of the anterior eye row (0.14 mm or more difference). In other words, the eyes in *Tigrosa* are spaced in a different geometric configuration than in *Hogna.* The epigynum of *H. radiata* (Fig. 39) is also distinct from the epigyna of species in *Tigrosa* (Figs. 9, 15, 18, 26, 33).

Color description.—*Female:* Dorsal pattern illustrated in Figs. 34. Face yellow-brown, darker brown along sides or in cheek area. Chelicerae dark reddish brown to black, eyes circled in black. Carapace brown with broad median cream to orange yellow stripe originating from PLE row and continuing to posterior edge. Median stripe as wide as the PME row throughout most of its length, widest in cephalie region and with dark lines radiating from it to submarginal stripes. Wide cream to yellow submarginal stripes with scalloped edges. Dorsum of abdomen brownish yellow to light brown. Dark brown cardiac mark bounded by lighter cream to yellow and with dark paired spots in posterior half. No discernible chevrons. Venter of abdomen dark brown to black from epigastric furrow to base of spinnerets; cream to pale brownish yellow along sides (Fig. 45). Legs yellow brown without darker markings on dorsal surfaces. Tibiae IV with definite proximal and distal dark bands on ventral surfaces. Tibiae III with less distinct bands in same positions. Labium and endites very dark brown to black with distal ends cream to yellow. Sternum and coxae very dark brown to black.

Male: Pattern illustrated in Fig. 35. Face yellow to pale brownish yellow without darker shading. Eye nacelles black. Carapace brown with dark lines radiating from yellow to orange yellow broad median stripe. Wide yellow to orange-yellow submarginal stripes with scalloped margins; outer margins sometimes reaching edge of carapace. Dorsum of abdomen brownish yellow with dark brown cardiac mark, outlined in pale yellow and with marginal dark spots halfway length of mark. Lighter regions of abdomen light brown. Venter of abdomen cream to yellow without darker markings. Legs light brown without darker bands on dorsal surfaees. Lighter yellow on ventral surface, with darker proximal and distal bands on tibiae IV. Labium and endites yellow without darker markings. Sternum and venter of coxae yellow.

Natural history.—The ecology and phenology described here is translated from Fuhn & Niculescu-Burlacu (1971). According to their studies of Romanian Lycosidae, *H. radiata* is a diurnal species that prefers warm, dry (xerophilic) habitats. It is found in dry grasses and open woods in steppes. In Romania adults were collected from March to December. Females with egg cases occur from August to December, and females with young on their back appear in December. In one case 138 spiderlngs were found with the female. *Hogna radiata* is active in the daytime, wandering through grass. Mating occurs in the fall and then the female digs a shallow burrow where she retreats to construct an egg case. The young leave the egg case in December, but spend the winter with their mother and disperse in April and May.

Distribution.—According to many reports in the literature, such as Roewer (1955), Fuhn & Niculescu-Burlacu (1971) and Platnick (2011), *Hogna radiata* occurs throughout many parts of Europe, eastern Asia and North Africa. Specimens from North Africa labeled *Hogna radiata* that I have examined are a different species than those I have seen from Spain, France, and Italy. Because of this and the fact that many species names have been applied to populations in different parts of the reported range, I suspect that more than one, and possibly several species, are now reported in the literature under *H. radiata.* I have focused upon specimens from France, which are nearer to the type locality for *H. radiata,* in comparing this species to *Tigrosa.*

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Predators and parasitoids of egg sacs of the widow spiders, *Latrodectus geometricus* and *Latrodectus hesperus* (Araneae: Theridiidae) in southern California

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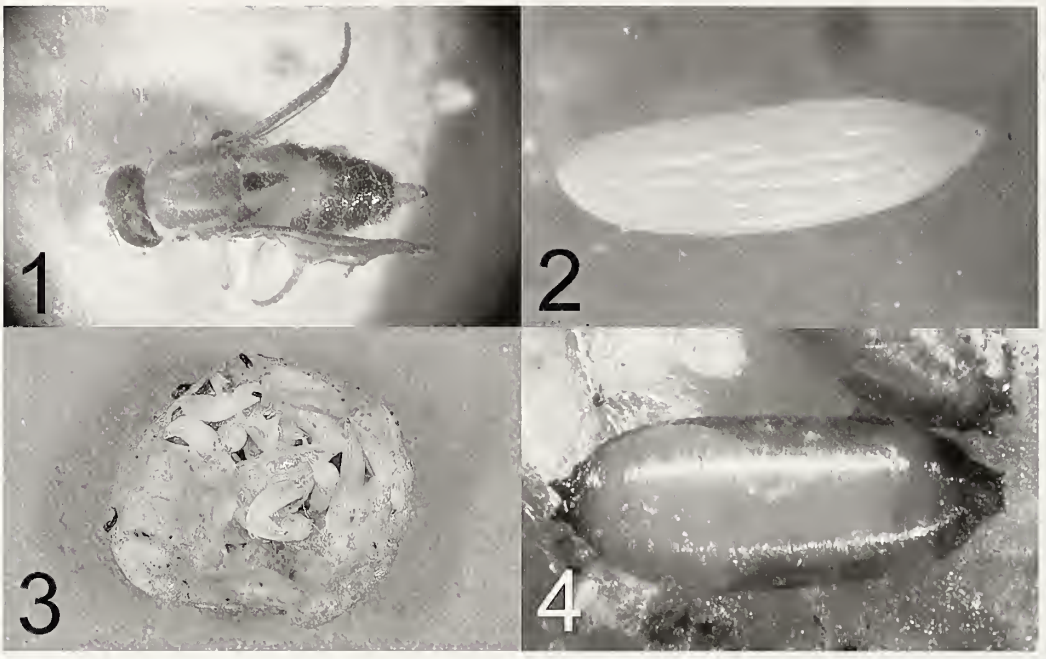
Abstract. The brown widow spider, *Latrodectus geometricus* C. L. Koch 1841, is non-native to North America and has experienced an explosive range expansion in the first decade of the 21st century. Previously restricted to peninsular Florida, it is now well established in the southeastern United States and southern California. In southern California, brown widow spiders have become ubiquitous around urban homes and are well known to the general public because of their high numbers and distinctive spiked egg sacs. Several insects attack egg sacs of the native western black widow, *L. hesperus* Chamberlin & Ivie 1935, as either parasitoids or egg predators. We investigated whether and to what degree these insects would attack brown widow egg sacs. We dissected 3,739 brown widow egg sacs finding evidence of the chloropid fly, *Pseudogaurax signatus* (Loew 1876) in 2.0% and wasp parasitoids in 0.4% of the sacs. For comparison, we also dissected 263 western black widow egg sacs with *P. signatus* showing a higher level of predation (6.1%). Other brown widow sac inhabitants included larvae and adults of dermestid beetles, psocids, and lepidopterans, which are probably scavengers or incidental occupants. The overall impact of the recorded predators and parasitoids is too low to explore the possibility of a biological control program. Additionally, due to the relatively low number of predators/parasitoids in brown widow egg sacs and the entanglement of small arthropods on the outer surface, we speculate that the spiked egg sac surface might serve as an effective barrier to most predators and parasitoids.

Keywords: Black widow, brown widow, egg predator, parasitism, *Pseudogaurax*

The brown widow spider, *Latrodectus geometricus* C. L. Koch 1841, was originally described from Colombia, South America (Lotz 1994) and is not native to North America. However, it is probably native to Africa where it has widespread distribution and has its closest sister species (Garb et al. 2004). It was first recorded in North America in Florida in 1935 (Pearson 1936) and was isolated in the peninsular portion of the state for decades. Inexplicably, in the first decade of the 21st century, an explosive population expansion occurred such that by 2011, it was well established in the southeastern United States from Texas to South Carolina (Brown et al. 2008; Vincent et al. 2008), with specimens reeently reported from North Carolina (pers. comm. to RSV). In southern California, the brown widow was first discovered in Los Angeles County in 2003 (Vincent et al. 2008), where it is now found in urban areas from San Diego to the western portions of Los Angeles County and east to western Riverside and San Bernardino Counties. In less than a decade, the brown widow has become one of the most common and easily recognizable spiders in urban southern California, such that the general public is both well aware of its presence and can usually accurately identify the spider or its egg sacs on their property.

The egg sac of the western black widow, *Latrodectus hesperus* Chamberlin & Ivie 1935, is known to be attacked by insect parasitoids and egg predators in southern California (Pierce 1942; Kaston 1970). A fly, *Pseudogaurax signatus* (Loew 1876) (Diptera: Chloropidae), (Fig. 1) lays its eggs (Fig. 2) on the outside of western black widow egg sacs; maggots hatch and push their way through the fibers of the sac to the inside where they feed upon multiple eggs and develop inside the sac (Kessel & Kessel 1937; Barnes et al. 1992). As they near pupation, the larvae (Fig. 3) thin the inside surface of the sac and pupate (Fig. 4), with emerging flies pushing their way through the thinned silk walls (Kessel & Kessel 1937). In contrast, the tiny wasp, *Baeus latrodecti* Dozier 1931 (Hymenoptera: Platygastridae s. l.) is an efficient egg endoparasitoid. The adult female chews its way into western black widow egg sacs and lays one egg per spider egg; within individual egg sacs, parasitism rates can approaeh 100% (Pierce 1942). *Baeus latrodecti* was discovered and reared in southern California for use in biocontrol programs in Hawaii against *Latrodectus unctatus* (Fabricius 1775); however, it was collected in only one isolated area in southern California (Pierce 1942). The females are 0.75 mm long and wingless, which may reduce their ability to disperse over wide distances, at least in southern California. From western black widow spider egg sacs in the San Francisco Bay area, Herms et al. (1935) found *P. signatus* and *B. latrodecti*, as well as an ichneumonid wasp, *Gelis* sp.

Pemberton & Rosa (1940) report that attempts to rear *B. latrodecti* on *L. geometricus* eggs failed; however, in a later study the same authors document an undescribed eurytomid wasp that was parasitizing brown widow egg sacs in Hawaii (Pemberton & Rosa 1946). Other eurytomid wasps that parasitize brown widow egg sacs include *Phlolema* (=*Eurytoma*) *arachnovora* (Hesse 1942), in Jamaica (Baerg 1954) and *Phlolema latrodecti* (Fullaway 1953) in Florida (Brown et al. 2008). Brown et al. (2008) note that they collected chalcidoid wasps in brown widow egg sacs, although they did not list the



Figures 1–4.—Chloropid fly, *Pseudogaurax signatus*. 1. Adult, removed from alcohol for photo, body length of 3 mm; 2. Egg, 0.55 mm long; 3. Larvae in a western black widow egg sac. This sac was opened, the larvae discovered and then photographed. In normal situations, the larvae remain inside the closed egg sac to pupate, and the adult flies emerge from the sac; 4. Pupa, 3.66 mm long.

location of collection. In southern Africa, Hesse (1942) lists *P. arachnovora* and adds *Gelis* (=*Pezomachus*) *latrodectiphagus* (Hesse 1942) as parasitoids of the African widow species, *L. indistinctus* O. P.-Cambridge 1904. In Australia, egg sacs of the red-back widow, *L. hasselti* Thorell 1870, are infested or attacked by two species of eurytomid wasps, one ichneumonid wasp, and a mantispid (Austin 1985).

Because the brown widow spider is a newly established non-native species, we were interested in determining the response of parasitoids and egg predators in southern California to this new potential host. This study was undertaken to determine 1) whether parasitoids and predators of western black widow eggs will attack brown widow egg sacs; 2) if so, to what level do they infest brown widow egg sacs and 3) can any of these egg predators or parasitoids be potential candidates for developing a natural biological control program to reduce or modulate the non-native brown widow populations?

METHODS

The egg sac of the brown widow is readily recognizable as a small sphere covered with silk spicules such that it has been described as looking like a large pollen ball or a World War II harbor mine (Fig. 5). In addition, it is common to find several to dozens of egg sacs in one location, hence increasing their conspicuousness to the average homeowner. Therefore, we were able to solicit egg sacs from homeowners throughout southern California, generating large numbers of sacs from a broader area than we would have been able to collect ourselves.

The project ran from May to October 2011. It was promoted on the Center for Invasive Species Research website at the University of California, Riverside. Because part of this study was funded by Orange County, we were allowed access to various county facilities (parks, zoos, horticultural facility, historic ranch) for searches. One of us (JNK) alerted the Orange County Master Gardeners to our project, requesting permission to search their property and gardens. In addition, articles in three local newspapers (Los Angeles Times, Orange County Register, Riverside Press-Enterprise), as well as interviews with two Los Angeles television stations and a radio station, instigated a great outpouring of specimen submissions by the general public. We also collected egg sacs of *L. hesperus* to provide a comparison to those of *L. geometricus*. Regarding the native western black widow egg sacs, 62% of the total were collected from two adjoining properties in Moreno Valley, California that were zoned for horses (i.e., there were barns with abundant western black widow habitat in xeric chaparral landscaping).

We pulled egg sacs open with fine forceps under a microscope and assigned the contents to various categories (Table 1). Minor overlap between some categories required us to establish sorting rules; that is, if an egg sac had about equal numbers of unhatched and cast shells of hatched eggs, we would place it in the Dead, Loose Eggs category but if there were just a few unhatched eggs but most hatched, we considered it to be in the Successful Hatch and Emergence category. In the category where egg or spiderling death was recorded, we could not differentiate among those that were dead due to infertility, insecticide, heat (ambient or encountered during shipping) or inability to chew an exit hole to escape. We recorded all instances of parasitism of egg sacs. Egg sacs that had live eggs or first or second instar spiderlings were placed in individual vials for later inspection in case there were still developing parasitoids or egg predators not visible amongst the hundreds of eggs. (In reality, only one of about 300 egg sacs treated this way resulted in the discovery of an egg predator.) We examined all egg sacs, no matter how old, because if parasitoids/predators were ever present therein, the evidence would still be detectable.

Figure 5.—Spiked egg sacs of the brown widow spider.

Most often the presence of *P. signatus* was determined by empty pupal cases. Although there is no key to pupal cases for this genus, of those sacs from which live flies emerged, all flies keyed out to *P. signatus* using Sabrosky (1966), and the empty pupal cases from other egg sacs matched those from which flies emerged. Hence, we assumed that there was only one species of fly preying on *Latrodectus* eggs and that all were *P. signatus*. Only two *Pseudogaurax* species exist in the United States with *P. anchora* (Loew 1866) being limited to the eastern U. S. and *P. signatus* having widespread distribution throughout the southern portion of the country (Sabrosky 1966). Hall (1937) mentions that *P. signatus* is found anywhere that *L. mactans* is found. (Note: in 1937, *L. mactans* was the blanket name used for several current *Latrodectus* species in the United States including *L. hesperus*.)

Egg sacs containing fly pupae or their empty cases were treated as follows to separate and harvest pupal cases that were intertwined in the interior silk of the egg sac. An egg sac was dipped into alcohol to reduce hydrophobicity of the silk and then placed into a small petri dish where it was covered with a small amount of commercial bleach (6% sodium hypochlorite), which dissolves spider silk in a few minutes (Vetter et al. 1996). The pupal cases were then easily extracted from the dissolved silk mass, counted, and placed in individual vials with alcohol and an identification label.

To understand the potential reproductive success of *P. signatus*, we counted the number of eggs in sacs for the brown widow (n = 30) and the western black widow (n = 10). We measured diameters of 40 eggs of each species with a Leica MZ16 microscope fitted with an ocular micrometer. Only field-collected egg sacs were used, so that the numbers that were generated would represent the productivity of naturally feeding females.

Rates of infestation for western black and brown widows were compared with an R × C contingency test of independence. The number of fly pupal cases and eggs per sac for each species was analyzed with a Welch's T-test for unequal variance. A comparison of the eggs per egg sac was analyzed with a two-sample t test (Statistix 9, 2009).

RESULTS

We dissected 3,739 brown widow and 263 western black widow egg sacs. Table 1 lists the contents of the sacs. The brown widow egg sacs showed an overall infestation rate of 2.38% including the chloropid fly, *P. signatus* (1.98%), parasitized eggs or empty capsules of small wasps (0.24%) and pupae or empty pupation capsules of larger wasps (0.16%), whereas the western black widow sacs showed an infestation rate of 6.1%, composed solely of *P. signatus*. Most of the wasp pupal capsules were empty, so it was not possible to assign predation/parasitism rates to specific species or to be assured that only one species was involved. The larger wasp pupal capsules were typically found attached to the outside wall of the brown widow egg sac or wedged between multiple spider egg sacs. It was assumed that these larger capsules represented wasps that were feeding on brown widow eggs, however it is possible that they merely used the egg sacs as a pupation substrate after feeding on another arthropod host nearby. Of the few wasps that emerged, wasp taxonomists identified males and females of *Aradophagus fasciatus* Ashmead 1893 (Platygastridae s. l.), and one male of *Gelis* sp. (Ichneumonidae). A lepidopterist colleague identified the Florida pink scavenger moth, *Pyroderces badia* (Hodges 1962) (Cosmopterigidae).

Table 1.—Results of the contents (by percentage) from dissections of 3,739 brown widow and 263 western black widow spider egg sacs for various categories.

	Brown widow	Western black widow
Fly Egg Predators or Wasp Parasitoids		
Fly pupae or empty pupal cases	1.98	6.08
Wasp parasitized eggs or empty pupal cases	0.40	0.00
Dead Material		
Dead, loose eggs (with any other content)	17.54	14.07
Dead spiderlings	4.23	2.66
Clump of dead, un-separated eggs	3.99	0.76
Live Material		
Live, viable-looking eggs	5.11	4.56
Pale, newly hatched 1st instars	2.41	0.38
Live mobile spiderlings	6.90	1.14
Egg sac empty, spiderlings dispersed		
Successful hatch and emergence	50.60	64.26
Sac scavenged, no remnant silk or exuviae	3.24	2.28
Other		
Dermestid beetle evidence	0.72	3.80
Amorphous blackened remnant (fungus?)	2.30	0.00
Other	0.56	0.00

When comparing the two spider species for the overall degree of *Pseudogaurax signatus* pupae presence, the western black widow had a significantly greater infestation rate than did the brown widow (G_1 = 13.36, $P < 0.005$). There was no statistical difference between the average number of fly pupae in western black widow egg sacs (10.63 ± 12.85, range 1–39, n = 16) and brown widow egg sacs (4.79 ± 4.39, range 1–25, n = 70) ($T_{15.8} = -1.79$, $P = 0.10$). (Because of the 2:1 ratio of fly pupae in black:brown widow egg sacs, we were surprised that this difference was not significant, but realized that it may have been due to low black widow sample size and great variance. In subsequent hypothetical calculations, assuming the same mean and variance for both spider species but increasing the black widow sample size to 30 or more, the

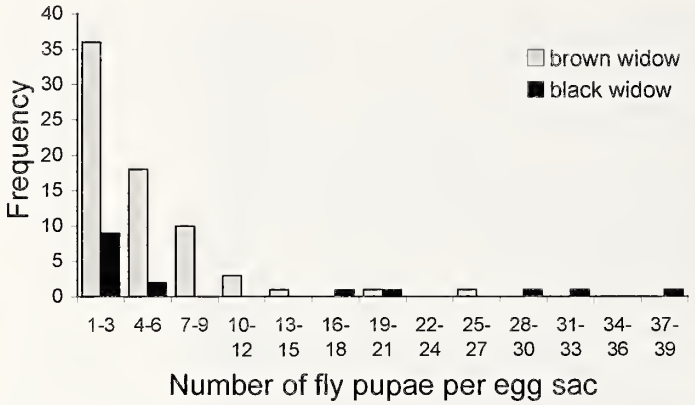


Figure 6.—The number of fly pupae found in brown and western black widow spider egg sacs.

difference would have been statistically significant.) The distribution of fly pupae was somewhat bimodal for western black widows with either few (< 6) or many (> 18) pupae found per sac; brown widow sacs produced few pupae (Fig. 6). The western black widow had a significantly greater number of eggs per sac (276.1 ± 97.2, range = 126–386, n = 10) than the brown widow (143.7 ± 61.6, range = 57–286, n = 30) (t_{38} = 4.68, $P < 0.0001$). Average egg diameters (and volumes) were 1.059 ± 0.026 mm (0.622 mm^3) for western black widows and 0.915 ± 0.023 mm (0.401 mm^3) for brown widows. Multiplying average egg volume by the number of eggs yields 171.7 mm^3 of egg volume in an average western black widow egg sac, compared to 57.6 mm^3 in an average brown widow sac, a ratio of 3.2 times more black than brown widow total egg volume available for egg predation.

In addition to the above-mentioned insects, we found evidence of other arthropods (Table 1), mostly scavengers or incidental occupants in the egg sacs. We found dermestid beetle larvae or their cast skins, empty lepidopteran pupal cases, and psocids. Dead Argentine ants, *Linepithema humile* (Mayr 1868), were occasionally found stuck to the outside of the brown widow egg sac and tangled in the loose threads among the silk spikes. Two brown widow egg sacs contained 74 and 15 ants of the genus *Monomorium* that had crawled inside, were dead and tangled in the inner sac silk. Interestingly, we found the shed skins of salticid spiders inside two empty sacs, indicating that they sought the interior of the sac as a safe refuge during molting.

We found a large number of egg sacs of both *Latrodectus* species that had unhatched, discolored and shrunken eggs, or dead first and second instars (Table 1). For the older spiderlings, it appeared that some of this resulted because of the inability of the spiderlings to chew an exit hole. In others, some sacs were completely empty inside; all evidence of eggs, shed skins and the inner layer of silk was gone. These empty sac walls often had a large (6 mm) hole as opposed to the small (0.8 mm) hole that the spiderlings chew to exit the sac. Occasionally, we found evidence of dermestids in these sacs, although a 6 mm hole is much larger than neeessary for a dermestid to enter and exit.

DISCUSSION

The current study examined thousands of brown widow egg sacs, virtually canvassing the entire southern California range where the spider currently is known to exist. The chloropid fly, *P. signatus*, infests brown widow egg sacs, albeit at a very low level. Additional parasitoids were found in extremely low frequency and, therefore, have insignificant potential for a biocontrol effect on brown widow populations. The one moth specimen that was identified (*Pyroderces badia*) may actually be an egg predator in addition to being a scavenger, as its common name indicates; in Australia, Austin (1977) doeumented *Pyroderces* (=*Anatrachyntis*) *terminella* Walker 1864 as an egg predator of the nephilid spider, *Nephila edulis* (Labillardière 1799). We saw no evidence of the tiny platygastrid wasp, *B. latrodecti*, attacking either spider species.

Pseudogaurax signatus was found in egg sacs throughout the southern California range of the brown widow, even though climate varies from the reliably temperate and humid coastal regions (such as La Jolla, Laguna Beach) to the hot, dry inland areas (Riverside, Redlands) where temperatures routinely exceed 40° C in summer. This is similar to the findings by Pierce (1942) where he reported this species in western black widow egg sacs throughout the Los Angeles Basin as well as from the desert cities of Coachella and Blythe where summer temperatures routinely reach 45° C and can approach 50° C on occasion. This fly also infests egg sacs of araneid and tetragnathid spiders (Pierce 1942; Barnes et al. 1992). Austin (1985) lists 26 spider species in eight families that are attacked by chloropid flies of the genera *Gaurax* or *Pseudogaurax*.

In our study, 6.1% of western black widow egg sacs were infested with *Pseudogaurax signatus*. Comparing this to other studies, *P. signatns* infested 40% of western black widow egg sacs in the San Francisco Bay area (Kessel & Kessel 1937) but only 4.8% in southern California (Pierce 1942). The low infestation rate of the brown widow egg sacs (1.98%) confirms that employing this fly for biological control is not promising in southern California. This is somewhat surprising given the great number of brown widow egg sacs that were available for predation compared to the lower number of western black widow sacs. However, there are many factors, both physical and chemical, that are involved in determining whether a parasitoid or predator can effectively switch hosts, a consequence of the tight evolutionary relationship between the species. Yet *P. signatns* has a somewhat eclectic host range, using several spider families, and was able to oecasionally utilize the non-native brown widow to some degree due to its recognition of a species in the *Latrodectus* genus. It might be interesting to revisit this relationship in five to ten years to see if *P. signatns* is able to increase its use of brown widow egg sacs for larval development over time. In contrast, host specificity for endoparasitoids may be more stringent. For example, even though the wingless wasp, *B. latrodecti*, is a very efficient parasitoid on *L. mactans* (Pemberton & Rosa 1940) and *L. hesperus* (Pierce 1942), it cannot be reared on *L. geometricus* (Pemberton & Rosa 1940). One additional avenue to pursue would be to examine the contents of mud-dauber wasp nests for brown widow spiders because immature black widows are favored prey of some species of the larger wasps (Irving & Hinman 1935; Rau 1935; Kaston 1970).

In contrast to the low levels of parasitism or egg predation, natural death of eggs inside the egg sac may account for much greater mortality. Many egg sacs contained eggs that never hatched, instead being dry and shriveled (Table 1). Baerg

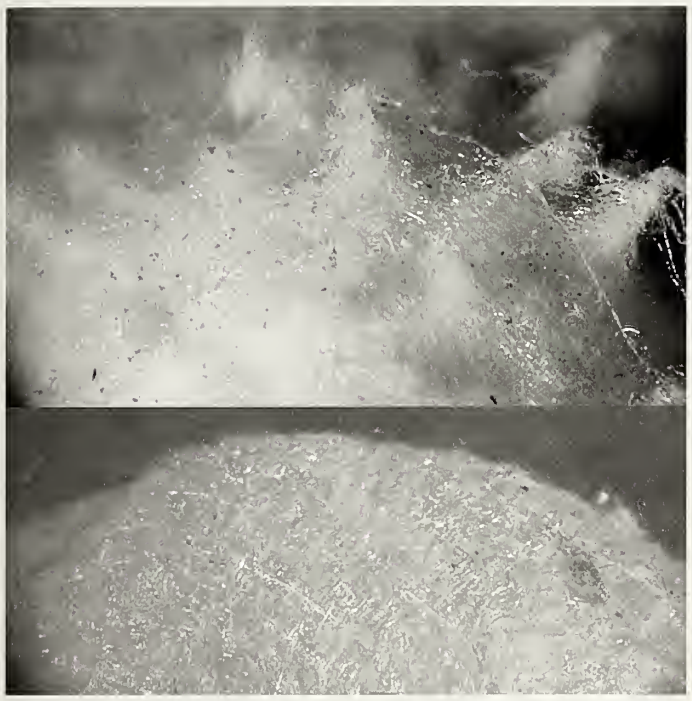


Figure 7.—Spiked egg sac surface of the brown widow spider (top) and the smooth egg sac surface of the western black widow spider (bottom).

(1954) mentions that a large percentage of brown widow egg sacs similarly did not develop during the drought season in Jamaica. Considering the Mediterranean climate in southern California, the hot inland temperatures might play a role in reducing brown widow populations or not allowing them to become established. However, the coastal regions are reliably cool and humid, which might encourage brown widow populations.

A very rewarding aspect of this study was the enthusiasm of the southern California homeowners in submitting egg sacs and allowing us access to their property. Piles of boxes came through the mail every week for most of the summer, and sometimes a homeowner spent several dollars to mail just one egg sac. This was similar to the experience of Pierce (1942) when he used the media to alert the Los Angeles public to the need for black widow egg sacs. In his article, Pierce acknowledges the help of many high school students who were critical in handling the large volume of western black widow egg sacs that were submitted. (One of these students was Evert Schlinger who eventually became Professor of Entomology at the University of California, Berkeley, researched acrocerid flies that parasitize spiders and served as major professor for many arachnology students including the second author of this paper.) Many times in our study during the collections at a home, the homeowner followed us around his or her yard as we collected, frequently pointing out spots where brown widows or their egg sacs had been seen. This was a rare, exceptional outreach moment where homeowners interacted with scientists and were both excited and pleased to contribute to a tangible, scientific study that might benefit them.

Egg sac construction may be influenced by the arms race of the spider host attempting to thwart attacks from parasitoids and predators, who in turn attempt to circumvent the host's defenses (Austin 1985). The two widow species had different infestation rates, which has instigated future research regarding the function of silk spicules on the surface of the brown widow egg sac. From the observation of small ants stuck to the outside of a sac and the significantly lower infestation rate of the brown widow sacs as compared to the western black widow, we postulate that the silk surface of the brown widow sac may act as an anti-predator/parasitoid defense. In addition to the silk spikes, there are strands of silk loosely traversing among the spikes. Small wasps might get entangled in or be deterred by the spikes and surrounding silk when they attempt to oviposit on or in the sac, whereas this seems less likely with the smooth surface of the western black widow egg sac (Fig. 7). *Pseudogaurax signatus* lay their eggs flat on the surface of black widow saes; possibly the rugged surface of the brown widow egg sac makes this a more difficult task or undesirable place for the fly egg predators to oviposit. Alternately or additionally, the spiked egg sac surface might entangle or delay newly hatched maggots that are looking for an entry point into the sac. The different infestation rates could also have been influenced by the fineness of the silk weave of the sacs. Brown widows are smaller than black widows, with brown widows possibly weaving a finer mesh, which makes it difficult for the *P. signatus* larvae to push their way inside the sac.

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Mark Hoddle (University of California, Riverside) invited RSV to create a brown widow spider website on the Center of Invasive Species Research website, and Mike Lewis (University of California, Riverside) installed the initial website and made multiple alterations as needed, for which we are grateful. Editor Linden Higgins and reviewer Andy Austin (University of Adelaide) made comments that improved the manuscript. We thank the hundred or so homeowners who very enthusiastically collected egg sacs and mailed them in for the study as well as the dozens of people who graciously allowed us to romp through their property, upending patio furniture and potted plants, crawling under decks, and inspecting nooks and crannies to extract spiders and egg sacs. We appreciate the assistance of the following taxonomists: Serguei Triapitsyn (University of California, Riverside) identified the *Aradophagus* wasp, Andrew Bennett (Canadian National Collection of Insects, Ottawa, Ontario) identified the *Gelis* wasp, and Jerry Powell (University of California, Berkeley) identified the *Pyroderces* moth. Larry Bednar (Bednar Consulting, Portland, Oregon) provided statistical assistance. The following Orange County agencies allowed us access to their facilities: Irvine Regional Park, Orange County Historic Parks Headquarters, University of California South Coast Field Station, Shadetree Nursery at University of California, Irvine, the Santa Ana Zoo and the Fullerton College horticultural yard. This study was funded in part by OC Parks, Orange County, California, the University of California Hansen Grant and the Schlinger Foundation.

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Abundance and web characteristics of *Micrathena gracilis* and *Micrathena mitrata* (Araneae: Araneidae) in west-central Illinois, USA

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Abstract. We investigated abundance and web characteristics (web elevation and spiral area) of the spiny orb weavers *Micrathena gracilis* (Walckenaer 1805) and *Micrathena mitrata* (Hentz 1850) using transect surveys in oak-hickory forest stands in west-eentral Illinois. Surveys resulted in 153 collected individuals or observations of adult females (70 *M. gracilis* and 83 *M. mitrata*). Peak abundance of both species oecurred in late July, with a density of 0.03 females per m^2 for each species. Web spiral area and web elevation were both greater for *M. gracilis* than *M. mitrata*. *Micrathena mitrata* web spiral area was larger in plots in which spiders had been previously removed than in plots without removal. These results suggest that the two species have different vegetation structure or microclimate preferences, and may respond to availability of unoccupied habitat differently.

Keywords: Web elevation, web spiral area, spiny orb weavers

The orb weaver genus *Micrathena* includes 104 species of primarily Neotropical forest spiders (Levi 1985). These spiders have a striking spiny abdomen and occupy vertical orbs with an open hub. They assume an upside-down position on the web, with the abdomen held horizontally (Gonzaga & Santos 2004). The smaller males are rarely found in the web with females (Levi 1985). Three species, *Micrathena gracilis* (Walekenaer 1805), *Micrathena mitrata* (Hentz 1850), and *Micrathena sagittata* (Walckenaer 1841) occur in the eastern United States, and all three are found in Illinois (Levi 1985; Sierwald et al. 2005).

Micrathena gracilis and *M. mitrata* co-occur in the deciduous forests of Alice L. Kibbe Life Science Station in west-central Illinois, USA. These two species share the same habitats and occur together during similar times of the year, with webs abundant in late summer and early fall (Howell & Jenkins 2004). Both species build their webs in the forest understory. Some *M. gracilis* individuals relocate frequently, but others may occupy the same web site for days or even weeks (Hodge 1987b). The orb portion of the web of *M. gracilis* is removed in the evening and rebuilt at dawn, but the frame of the web may persist for several days (Hodge 1987a). *Micrathena gracilis* is the larger of the two species, with females ranging from 7.0 to 10.8 mm and males 4.2 to 5.1 mm in length. *Micrathena mitrata* females range from 4.7 to 6.0 mm and males from 3.0 to 3.7 mm in length (Levi 1978).

Polyphagous predators can be ecologically important in influencing prey populations, as evidenced by successful biological control programs involving generalist predators (Murdoch et al. 1985; Riechert & Lawrence 1997). Several studies have investigated *M. gracilis* natural history and behavior, including prey selection, attraction, and web orientation (Uetz & Biere 1980; Biere & Uetz 1981; Uetz & Hartsock 1987; Vanderhoff et al. 2008), mating behavior (Bukowski & Christenson 1997a, b, 2000), and web site residence time and macrohabitat selection (Hodge 1987a, b). However, there is relatively little information on *M. mitrata* specifically and on the two species when co-occurring. In this study, we compared abundance and web characteristics (web spiral area and web elevation) of *M. gracilis* and *M. mitrata*.

METHODS

We did this study at Alice L. Kibbe Life Science Station in Hancock County, Illinois, USA. We established four study plots (40°21′59.01″N, 91°24′30.53″W; 40°22′00.75″N, 91°24′23.18″W; 40°22′10.37″N, 91°24′35.46″W; 40°22′10.85″N, 91°24′31.02″W) in a mature dry-mesic upland oak-hiekory forest. Each plot consisted of two transects. Each transect was 80 m^2 (4 m wide and 20 m long), and transects within plots were 15 m apart. Plots were ca.100 to 150 m apart, and were a minimum of 50 m from the forest edge.

We surveyed for *Micrathena* between 09:00 and 15:00 on 11 dates (29 May, 10 June, 13 July, 22 July, 29 July, 11 August, 23 August, 13 September, 20 September, 11 October and 20 October) by walking the transects and scanning vegetation for the presence of *Micrathena* females. We misted the webs with a water bottle to make them more visible (Tolbert 1977). For eaeh *Micrathena* web found, we recorded height and width of the web spiral and used these measurements to calculate web spiral area. We also measured web elevation (distance to the bottom of the web spiral from the ground). In two plots (the "sampled" plots) we collected the *Micrathena*, and in the other plots (the "observation" plots) we identified the species of *Micrathena* but did not collect them. This was done to examine potential effeets of removal on *Micrathena* web characteristics.

Mean web spiral area and elevation, with 95% confidence intervals, were calculated for sampled and observation plots, and for the pooled data, for each *Micrathena* species. Because no spiders from the sampled plots had been removed prior to 13 July, calculations were also done excluding the data from this date. We tested for a potential association between relative abundance of the two species and survey method using the chi-square test with the 13 July data excluded.

Voucher specimens are deposited in the Western Illinois University Department of Biological Sciences Entomology Collection.

Table 1.—*Micrathena gracilis* and *Micrathena mitrata* mean web spiral area (cm^2) and elevation (cm), with 95% confidence intervals in parentheses, in sampled (spiders were collected) and observation (spiders were recorded but not collected) plots, and pooled across survey method, in Hancock County, Illinois, oak-hickory forest. For *M. gracilis*, $n = 70$ ($n = 30$ and $n = 40$ in sampled and observation plots, respectively). For *M. mitrata*, $n = 83$ ($n = 41$ and $n = 42$ in sampled and observation plots, respectively).

Web characteristic	Survey method	*Micrathena gracilis*	*Micrathena mitrata*
Spiral area	Sampled	438.2 (345.6, 530.8)	239.7 (198.0, 281.3)
	Observation	405.1 (350.1, 460.1)	170.9 (149.9, 191.9)
	Pooled	419.3 (370.0, 468.7)	204.9 (181.0, 228.7)
Elevation	Sampled	147.6 (135.2, 159.9)	100.7 (88.5, 112.9)
	Observation	155.3 (149.8, 160.8)	105.9 (97.6, 114.2)
	Pooled	152.0 (145.9, 158.0)	103.3 (96.1, 110.5)

RESULTS

Our study produced 153 collected individuals or observations of adult female *Micrathena* sp. (70 *M. gracilis* and 83 *M. mitrata*). We found the first adult females of both species on 13 July. Greatest abundance of *M. gracilis* (21 individuals) occurred on 29 July whereas greatest abundance of *M. mitrata* (also 21 individuals) occurred on 22 July, giving a maximum density of 0.03 females per m^2 for each species. We found female *M. gracilis* as late as 20 September and *M. mitrata* until 11 October.

Thirty (42.9%) of the *M. gracilis* females and 41 (49.4%) of the *M. mitrata* females were found in the sampled plots. Excluding the 13 July data, 26 of 60 (43.3%) *M. gracilis* and 37 of 72 (51.4%) *M. mitrata* were found in sampled plots. There was no statistically significant difference in relative abundance of the two species in relation to the survey method ($X^2_1 = 0.85$, $P = 0.36$).

Mean web spiral area was greater for *M. gracilis* than for *M. mitrata*, based on lack of overlap in confidence intervals (Table 1). Neither *M. gracilis* mean web spiral area nor mean web elevation differed between sampled and observation plots. *Micrathena mitrata* webs had greater mean web spiral area in sampled than in observation plots (Table 1). Exclusion of 13 July data changed these means and confidence intervals slightly but did not affect overall results.

DISCUSSION

Micrathena gracilis and *M. mitrata* were present in roughly equal numbers based on our survey results, and seasonal patterns of the two species overlapped substantially. Some *Micrathena* individuals may occupy the same web site for extended periods, though this behavior has only been studied in *M. gracilis*, for which a mean residence time of 6.7 d was found (Hodge 1987b). It is therefore possible that we surveyed some individuals more than once in the observation plots, although the length of time between survey dates (minimum = 7 d, mean = 15.4 d) probably minimized this.

We found little overlap in either web spiral area or web elevation between the two species, which could contribute to resource partitioning. Differences in web spiral area and elevation may also reflect differences in microhabitat preferences. Hodge (1987a) suggested that vegetation structure, which determines the spatial structure of attachment sites, is important in habitat selection by *M. gracilis*. The lower elevation of *M. mitrata* webs in our study could be related to availability of suitable attachment sites for their smaller webs. Intense solar radiation has been shown to affect web orientation or body position in *M. gracilis* (Biere & Uetz 1981) and *Micrathena schreibersi* (Perty 1833) (Robinson & Robinson 1974). The lower elevation of *M. mitrata* webs in our study could be associated with avoidance of direct sunlight as well. Competition may also be a factor in determining habitat use patterns. Uetz et al. (1978), in a study of central and southern Illinois *Micrathena*, showed that presence of other species, especially congenerics, can result in changes in *M. gracilis* web placement. However, unlike our study, Uetz et al. (1978) found that *M. gracilis* web elevation overlapped that of *M. mitrata* substantially, and *M. mitrata* upper elevation limits were greater than those of *M. gracilis*. Webs of *M. sagittata* tended to occur at lower elevations in that study. This suggests that relative elevation of webs of these species may vary with habitat, geographic location, or presence of another congeneric.

Webs of *M. mitrata* were substantially larger in the sampled than in the observation plots in our study (Table 1). This could indicate that *M. mitrata* web size is constrained by the presence of *M. gracilis*, but that *M. mitrata* invades available habitat more quickly than *M. gracilis* and occupies more favorable web location sites in the absence of its congeneric. Our results could also suggest greater web size plasticity in *M. mitrata* than in *M. gracilis*.

These results indicate that *M. gracilis* and *M. mitrata* are abundant orb weavers in west-central Illinois upland forests and differ substantially in web elevation and spiral area. The two species may also differ in their response to availability of unoccupied habitat and in their microhabitat requirements.

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An experimental study of spiders in a shrub-steppe ecosystem: the effects of prey availability and shrub architecture

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Abstract. Habitat structure is of great importance for the distribution and abundance of various organisms. Spiders are especially sensitive to structural features of their environment. Although spiders are influenced by habitat structure, it remains unclear whether spiders respond to architecture, to differences in prey availability associated with different architectures, or both. Here, we investigated the effects of shrub architecture and prey availability and their interactions on a spider community in a shrub-steppe environment in northern Utah, USA. Big sagebrush shrubs, matched by size, were randomly assigned to six experimental treatments: two levels of prey attractant (shrubs were either baited or not baited) and three levels of foliage density (low, natural/control, or high). We found that spider abundance and species richness were affected by both prey availability and shrub architecture, while variation in spider species diversity (Shannon-Wiener index) was governed by changes in shrub architecture. Spider species and family compositions were also associated with changes in shrub architecture, although guild composition was not. We discuss the implications and limitations of these findings and present suggestions for future research.

Keywords: Habitat structure, spider prey, Araneae, sagebrush

Ecologists have long been interested in patterns of community structure and the mechanisms that generate these patterns (Hutchinson 1959). Community structure is the result of interactions among many factors, making it difficult to assess the relative contribution and importance of any one factor (Uetz 1991). Clearly, if we are to understand and manage communities, there is a need to disentangle the different ecological factors that shape their composition.

Habitat structure, defined as the physical composition and arrangement of objects in space and time, is one of several factors considered important in influencing the distribution and abundance of animals (McCoy & Bell 1991). Structurally complex habitats provide animals with a wider array of microhabitats, more diverse ways of exploiting food resources, amelioration of climatic extremes, and protection from predators (see reviews in Bell et al. 1991). Habitat structure influences a variety of organisms, including birds (MaeArthur & MacArthur 1961), lizards (Pianka 1966) and various invertebrates (Lawton 1983), including spiders (Uetz 1991; Wise 1993).

Spiders are influenced by several structural attributes of the environment, including vegetation density and height (Hatley & MacMahon 1980; Abraham 1983; Brierton et al. 2003), as well as interactions among variables such as branch height and orientation (Heikkinen & MacMahon 2004). Spiders may even distinguish between different branch types, with some spiders being more common on reproductive than on vegetative branches (de Souza & Martins 2004).

Although spider communities differ with changes in habitat architecture, it remains unclear whether spiders are responding to architecture per se or to differences in prey availability caused by different architectures. Although some studies suggest that prey availability is important in understanding patterns of spider community structure (Riechert 1974; Horváth et al. 2005), others emphasize that prey availability is of lesser importance and that spider communities are shaped primarily by habitat structure (Greenstone 1984; Halaj et al. 2000; Chan et al. 2009). These findings highlight the need to further evaluate the processes responsible for structuring spider communities.

Our goal for this study was to investigate the relative importance of prey availability and shrub architecture in determining the composition of a well-studied spider community in a shrub-steppe environment in northern Utah, USA. Spiders are model organisms for addressing ecological studies. They are ubiquitous, locally abundant, taxonomically diverse, and amenable to experimental manipulations (Hatley & MacMahon 1980; Wise 1993). Spiders are especially well-suited for investigating the effect of shrub architecture on community organization because, as carnivores, they are not directly reliant on a particular plant species as a food source (Hatley & MacMahon 1980) and, for web-builders, the building of a web often requires specific substrates for attachment (Uetz 1991).

METHODS

Study site.—Our research expands upon earlier studies of spider communities in the Great Basin shrub-steppe ecosystem of northern Utah (Hatley & MacMahon 1980; Robinson 1981; Abraham 1983; Wing 1984; Ehmann & MacMahon 1996; Heikkinen & MacMahon 2004). This study was conducted at Hardware Ranch Wildlife Management Area (41.61° N, 111.57° W). Hardware Ranch WMA is located in the Wasatch-Cache National Forest, about 40 km southeast of Logan, Cache County, Utah and is managed by the Utah Division of Wildlife Resources. The site is at an elevation of 1731 m and is dominated by big sagebrush (*Artemisia tridentata*) and low sage (*Artemisia arbuscula*). The land is used primarily as winter range for big game.

Shrub selection.—To reduce the heterogeneity among individual shrubs, we applied several criteria when selecting shrubs. Experimental shrubs (*A. tridentata*) had a single trunk at ground level, were not in immediate contact with an adjacent shrub and were at least 10 m from another

experimental shrub. We measured shrubs before and after treatment for maximum canopy width, width perpendicular to maximum canopy width and canopy height (excluding the trunk beneath) (Hatley & MacMahon 1980). Only shrubs with all three canopy dimensions between 0.4 and 1 m were selected. Shrub volume was determined by using the formula for an ellipsoid

$$volume = 4/3\ \pi\ a\ b\ h$$

where a and b represent, respectively, the linear dimensions of the major and minor axes, and h represents height.

Study design and treatments.—We permanently identified shrubs selected for study with a numbered tag to facilitate location and data collection and then randomly assigned them to six experimental treatments, with 25 replieates per treatment. Experimental treatments consisted of factorial combinations of two levels of prey attractant and three levels of foliage density. Prey attractant treatments included shrubs that were either baited or not baited. The purpose of the bait was to inerease the probability of prey visits and/or the duration of each visit (Wing 1984). Baited shrubs contained four suspended containers: two (59 ml) containers filled with pig offal, one (22 ml) container filled with yellow banana-oil flavored honey, and one (22 ml) container filled with red-colored honey. Container lids were perforated to facilitate odor dispersion. As a control, identical but empty containers were suspended from shrubs not baited. We baited shrubs two weeks prior to sampling to maximize arthropod abundance on shrubs (Robinson 1981).

Shrub architecture was manipulated to either increase or decrease shrub foliage density (Hatley & MacMahon 1980). We increased foliage density by tightly binding all branches together (hereafter referred to as "high") and decreased density by clipping shrub foliage ("low"). Shrubs not manipulated were used as controls ("natural"). Shrubs were manipulated in spring of 2007 and 2008. We calculated differences in shrub foliage density using photographs taken from a digital camera (Nikon Coolpix L12) positioned approximately 1.5 m from the shrub. A white cloth attached to a wooden frame (1.5 × 1.5 m) was positioned behind the shrub and before and after treatment pictures were taken. Pictures were taken again at the end of the first sampling season. The pictures were imported into Adobe Photoshop CS4. Here, shadows surrounding the shrub were first removed using the 'color range' option. Images were then transformed into a black and white image by means of the 'threshold' option and the area occupied by the shrub was outlined using the magnetic 'lasso' tool. The 'histogram' tool was then used to determine the ratio of white (background) vs. black (vegetation) pixels. For each picture, this procedure was carried out twice and the average was taken.

Determination of sampling effort.—Before experimental manipulations, we sampled fifty randomly chosen shrubs to obtain a preliminary survey of the spider community. A species accumulation curve was then generated. Species accumulation curves show the rate at which new species are found by plotting the cumulative number of observed species as a function of sampling effort (Magurran 2004). As sampling efforts increase and as fewer new species are found, the curve approaches an asymptote, indicating that a representative sample was achieved given the collection method used. Here, we determined that a sampling effort of 25 shrubs per treatment combination was sufficient. Species accumulation curves were generated using the 'specaccum' function in the 'vegan' package (Oksanen et al. 2010) of R environment (R Development Core Team 2011).

Sampling of arthropods.—We sampled shrubs during a five-day sampling period once a month in June, July, August and September of 2007 and 2008. September samples from both years and a few samples from the remaining collections were discarded because of bait disturbances. Sampling periods took place at intervals of no less than three weeks. Sampling began approximately two hours after sunrise, occurred only when there was an absence of high winds and precipitation, and did not occur when temperatures were below 10 °C. We collected arthropods by using the beating technique (Ehmann & MacMahon 1996). Each shrub was quickly surrounded at the base with a canvas sheet (1.5 × 1.5 m) and then beaten 15 times with an ax handle to dislodge specimens onto the beating sheet for collection. Specimens were collected with an aspirator and immediately preserved in vials containing 70% ethanol. After the arthropods from the first beating were collected, a second beating episode of the same duration followed. The double-beating method was used previously and resulted in a 100% collection rate (Ehmann & MacMahon 1996).

Since this sampling technique may emphasize sedentary prey while ignoring highly active prey, sticky traps were also used to monitor prey availability. A sheet of clear plexiglass (25 × 25 cm) was coated on both sides with Tanglefoot® trap coating (Tanglefoot Co., Grand Rapids, MI) and attached to two vertical stakes (Greenstone 1984; Halaj et al. 2000). During July of 2007, we placed one trap next to each of five randomly chosen shrubs from each treatment type not sampled by the beating technique. Each trap was positioned 20 cm from a given shrub, and the cardinal direction of the trap was determined at random. After five days, the traps were collected and taken to the laboratory (Wing 1984). These traps may not mirror suitable prey or the exact resource base available to spiders, but they do allow for the analysis of specimens active at a given time and place (Rypstra 1986).

We identified spiders to species and measured their body length (not including spinnerets) to the nearest 0.1 millimeter. We excluded immature spiders from analyses, since their behavior and habitat may differ from adults, but also because some immature spiders were difficult to identify to species (Sacket et al. 2008).

We further sorted spiders into a priori guilds, or groups of organisms that exploit the same resource in similar ways (Root 1967). These assignments are user-defined parameters widely used in community studies (Hawkins & MacMahon 1989). For spiders, guild membership is based on observations of foraging techniques that are often reinforced by morphological characteristics shared at the family level (Post & Riechert 1977). However, since there are no absolute guidelines, spider guild assignments vary widely (Uetz et al. 1999). In this study, two different approaches for the classification of spider foraging guilds were used. Following the classification proposed by Uetz et al. (1999), we grouped spider families into the following four guilds: 1) ambushers: Philodromidae and Thomisidae; 2) runners: Gnaphosidae and Lycosidae; 3)

stalkers: Mimetidae, Oxyopidae and Salticidae; and 4) trappers: Araneidae, Dictynidae, Linyphiidae and Theridiidae. The second approach followed the classification commonly used for spiders on big sagebrush (Hatley & MacMahon 1980; Robinson 1981; Wing 1984; Heikkinen & MacMahon 2004), where members from the family Philodromidae were analyzed as runners instead of ambushers. Relationships between spider hunting strategies and spatial characteristics of the vegetation have previously been described. In general, ambushers prefer dense foliage, stalkers and trappers prefer open foliage, and runners prefer a variety of foliage types (Hatley & MacMahon 1980; Uetz et al. 1999).

We identified potential prey items to the order level or below and assigned them to the following functional groups: detritivores, herbivores (including pollinators) and natural enemies (predators and parasites/parasitoids). Prey composition was examined to assess whether differences among treatments, if present, correspond to variations in spider community structure. Taxonomic classification followed Triplehorn & Johnson (2005), and functional group assignments were based on dietary information provided also by Triplehorn & Johnson (2005). We did not collect ants (Hymenoptera: Formicidae) or aphids (Hemiptera: Aphididae) because their high abundanees made collection of samples in a short period of time difficult. All specimens were deposited in the Department of Biology at Utah State University for reference.

Data analyses.—We compared mean shrub foliage density among treatments with a repeated measures one-way analysis of variance (ANOVA). Relevant pairwise comparisons were made as needed and family-wise Type I errors were controlled by applying the Tukey-Kramer method. An unstructured covariance matrix was selected to model repeated measures across the three measurements based on Akaike's Information Corrected Criterion (AIC_C). A two-way ANOVA, with foliage density and prey attractant treatments as factors, was used to analyze square-root transformed sticky trap data. The ANOVAs were performed using the MIXED procedure in SAS/STAT software Version 9.2 in the SAS System for Windows (SAS Institute 2011).

We tested the effects of foliage density and prey attractant treatments on spider and potential prey abundance, as well as spider species diversity and richness, using a general linear mixed model (LMM) with repeated measures. Spider diversity was determined using the Shannon-Wiener diversity index (Magurran 2004), and spider and potential prey abundances were converted into densities (individuals per m^3) to account for differences in shrub volume. Experimental treatments were treated as fixed factors, while shrubs were incorporated in the model as a random effect and treated as independent replications. An unstructured covariance matrix was used to model repeated measures across three months in each of two years. Response variables were ln-transformed ($x + 1$) to improve model performance. For main effects, pairwise mean comparisons were adjusted for family-wise Type I errors using the Tukey-Kramer method. Pairwise comparisons for significant interaction terms were examined with stepdown Bonferroni adjustments. Analyses were carried out using the MIXED procedure in SAS/STAT software (SAS Institute 2011).

Experimental foliage treatments did not produce shrubs of equal density within each treatment group. Likewise, prey density varied among shrubs within a treatment group. Hence, because continuous variables may be more informative than discrete levels, we also analyzed data using regression analyses. Spider density, diversity, and richness were regressed on continuous measures of foliage density and prey density using multiple linear regression, and prey density was regressed on foliage density using simple linear regression. Since foliage densities were not measured consecutively across sampling periods, spider and prey densities were averaged for shrubs sampled during all sampling periods. Natural-log transformations were applied to averaged spider and prey densities to satisfy statistical assumptions. Regression analyses were performed using the REG procedure in SAS/STAT software (SAS Institute 2011).

To test the hypothesis that spider and potential prey community composition differed among experimental treatments, we used a permutational multivariate analysis of variance (PERMANOVA) (Anderson 2001). PERMANOVA differs from traditional multivariate analysis of variance (MANOVA) by relaxing the assumptions of a multivariate normal distribution. Computations were performed using the 'adonis' function in the 'vegan' package of R environment (R Development Core Team 2011), and significance values were generated using 1000 permutations (Oksanen et al. 2010). We then used a similarity of percentages (SIMPER) analysis to determine which taxa contributed to overall differences in community composition. Taxa contributing $\geq$ 5% to the between group dissimilarities were highlighted. SIMPER tests were carried out using the program PRIMER v. 6 (Clarke & Gorley 2006).

We illustrated differences in compositional patterns with non-metric multidimensional scaling (NMDS) plots using the 'metaMDS' function in the 'vegan' package of R (R Development Core Team 2011) (Oksanen et al. 2010). NMDS arranges objects (i.e., sites) in multidimensional space so that points in close proximity are more similar (e.g., in species composition) than those further apart. NMDS is considered to be one of the most robust ordination techniques available because it is well suited for non-normal data and does not assume linearity between species and environmental gradients (McCune & Graee 2002).

Multivariate analyses were performed using pooled densities for shrubs sampled during all sampling periods. Prior to analyses, data were square-root transformed to reduce the influence of the most abundant taxa, then standardized by sample (i.e., shrub) to minimize differences in total abundance (McCune & Grace 2002). Distance matrices were calculated using the Bray-Curtis dissimilarity index, and taxa represented by less than 10 individuals were removed from the data set (McCune & Grace 2002).

Significant differences in results refer to a statistical significance of $P \leq 0.05$. Unless otherwise specified, data are presented as mean $\pm$ standard error.

RESULTS

Shrub manipulations.—Architectural treatments were designed to modify foliage densities. Shrub foliage densities were similar among treatment groups prior to experimental manipulations (ANOVA, $F_{2,147} = 0.5$, $P = 0.58$). Following manipulations, low and high foliage density shrubs were

different from their initial foliage densities; and foliage densities for each architectural treatment were different from the other two treatments, with differences persisting at the end of the sampling season (all $P < 0.001$). Low foliage density shrubs averaged a 13.5% loss of density (i.e., vegetation pixels), while high foliage density shrubs showed an 8.4% gain in density.

Potential prey density and community composition.—A total of 9929 potential prey were collected, representing 15 orders and more than 66 families (see Appendix 1). Leafhoppers (Hemiptera: Cicadellidae), plant bugs (Hemiptera: Miridae) and leaf beetles (Coleoptera: Chrysomelidae) comprised over 77% of the non-Araneae arthropods collected.

Potential prey densities were influenced by the interaction between foliage density and prey attractant (LMM, $F_{2,125} = 3.5$, $P = 0.035$). With the exception of natural foliage density shrubs, baiting shrubs did not suceeed in changing the prey base. Low and high foliage density shrubs contained fewer prey items with the introduction of prey attractant, while natural foliage density shrubs contained more prey when shrubs were baited than when they were not (Fig. 1A). In addition, the main effect of prey attractant was not statistieally significant (LMM, $F_{1,125} = 0.02$, $P = 0.90$), although the main effect of foliage density was highly significant (LMM, $F_{2,125} = 17.6$, $P < 0.001$). More prey items were collected in high foliage density shrubs than in natural or low foliage density shrubs, and natural foliage density shrubs contained more prey than low foliage density shrubs. Prey densities were also influenced by the interaction between year and month of data collection (LMM, $F_{2,127} = 60.6$, $P < 0.001$). Prey densities declined from June to August of 2007, but were similar across months in 2008 (Fig. 1B). A simple regression analysis also revealed an influence of foliage density on prey density (regression equation: $\ln(y) = 1.333 + 0.034$ (foliage density), $R^2 = 0.12$, $P < 0.001$). Lastly, sticky traps did not detect significant differenees in potential prey densities among foliage density and prey attractant treatments (ANOVA, main effects and interaction, $P > 0.1$). Only one spider was collected from the sticky traps.

Potential prey community composition did not differ among foliage density and prey attractant treatments, either at the level of orders or by functional group (PERMANOVA, main effects and interaction, $P > 0.1$).

Spider density, diversity, and community composition.—A total of 6262 spiders were collected, of which 4518 (72%) individuals were immature. Of adult specimens, 31 speeies were collected (see Appendix 2). Members from the family Salticidae were numerically dominant (48%), followed by Philodromidae (21%), Dictynidae (9%), Oxyopidae (8%) and Theridiidae (6%). Families Araneidae, Gnaphosidae, Linyphiidae, Lycosidae, Mimetidae and Thomisidae were also collected, although in fewer numbers. The five most abundant species were *Pelegrina clemata* (Levi & Levi 1951) (Salticidae), *Philodromus histrio* (Latreille 1819) (Philodromidae), *Ebo pepinensis* Gertsch 1933 (Philodromidae), *Oxyopes scalaris* Hentz 1845 (Oxyopidae) and *Emblyna reticulata* (Gertsch & Ivie 1936) (Dictynidae); which together characterized 70% of the adult spiders collected.

Spider densities were influenced by foliage density treatment (LMM, $F_{2,139} = 22.1$, $P < 0.001$). More spiders were collected in high foliage density shrubs than in natural or low foliage

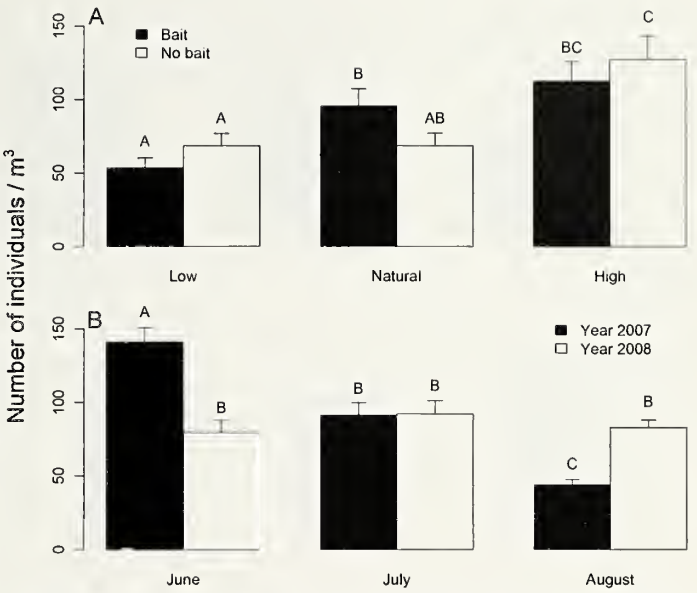

Figure 1.—Potential spider prey densities sorted by A) different foliage density and prey attractant treatments and B) year and month of collection. Graphs show means with standard errors. Different letters indicate a significant differenee at $P < 0.05$. Means and standard errors were back-transformed from ln-transformed estimates.

density shrubs, and natural foliage density shrubs contained more spiders than low foliage density shrubs (Fig. 2A). A multiple regression analysis showed that spider density was associated with both foliage density and prey density ($P = 0.005$ and < 0.001, respectively) (regression equation: $\ln(y) = -1.557 + 0.023$ (foliage density) $+ 0.502 \cdot \ln$ (prey density), $R^2 = 0.34$), although the LMM main effect of prey attractant treatment on spider densities was not significant ($F_{1,139} = 1.0$, $P = 0.31$), nor was the interaction between the two factors ($F_{2,139} = 1.7$, $P = 0.19$). Spider density was also influenced by year and month of data collection (LMM, $F_{2,138} = 4.1$, $P = 0.018$). Spider densities declined from June to August of 2007, but were static across months in 2008 (Fig. 2B).

Spider species diversity (H′) differed by month of collection (LMM, $F_{2,114} = 8.0$, $P < 0.001$) and by foliage density treatment (LMM, $F_{2,108} = 3.1$, $P = 0.048$). Spiders reached their highest diversity in June (mean Shannon index ± SE: 0.90 ± 0.03), followed by July (0.77 ± 0.03) and August (0.77 ± 0.03). Spiders were also more diverse on high and natural foliage density shrubs (0.86 ± 0.01 and 0.82 ± 0.07, respectively) than on low foliage density shrubs (0.75 ± 0.02). A multiple regression analysis showed that spider diversity was associated with foliage density ($P < 0.001$), but not with prey density ($P = 0.24$) (regression equation: $y = -0.471 + 0.01$(foliage density), $R^2 = 0.13$).

Spider species richness was influenced by year and month of collection (LMM, $F_{2,140} = 4.9$, $P = 0.009$), as well as foliage density treatment ($F_{2,139} = 15.4$, $P < 0.001$). More species were collected during June (mean number of species ± SE: 6.62 ± 0.09) than July (6.20 ± 0.07) and August (6.14 ± 0.06), with species richness being higher in June 2007 (6.90 ± 0.12) than in June 2008 (6.35 ± 0.11). More species were also collected on natural and high foliage density shrubs (6.63 ± 0.10 and 6.42 ± 0.10, respectively) than on low foliage density shrubs (5.93 ± 0.09). A multiple regression analysis revealed

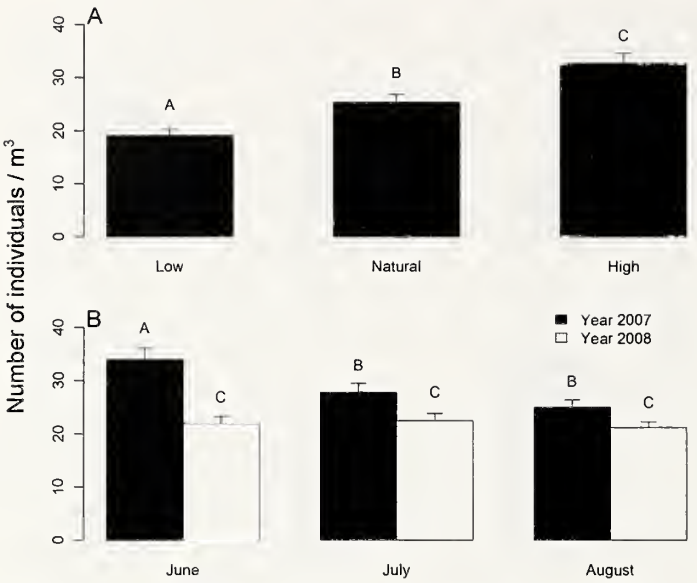

Figure 2.—Spider densities sorted by A) different foliage density treatments and B) year and month of collection. Graphs show means with standard errors. Different letters indicate a significant difference at $P < 0.05$. Means and standard errors were back-transformed from ln-transformed estimates.

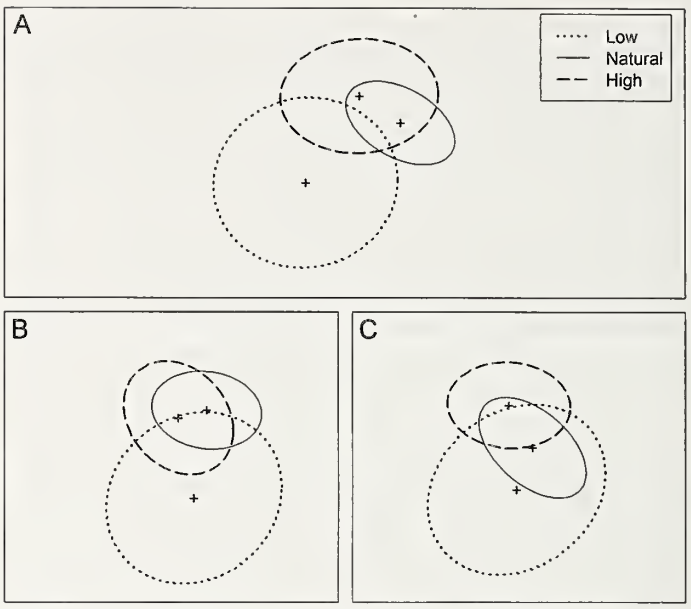

Figure 3.—Non-metric multidimensional scaling (NMDS) plots representing variation in A) spider species composition, B) spider family composition, and C) spider guild composition, where guild composition followed the classification proposed by Uetz et al. (1999). Foliage density is plotted as centroids (+ symbols) and 95% confidence ellipses of the mean sample score. Confidence ellipses are for visualization only; actual significance tests were obtained from PERMANOVA analyses (see Table 1 for R^2 and significance values). Final stress for a two-dimensional (2D) solution was 21.66 for the species ordination, 21.48 for the family ordination, and 11.25 for the guild ordination.

that spider species richness was related to both foliage density and prey density ($P = 0.012$ and 0.001, respectively) (regression equation: $y = -1.244 + 0.02$ (foliage density) + 0.262•ln (prey density), $R^2 = 0.17$).

Spider species composition varied with foliage density (Table 1, Fig. 3A). A SIMPER analysis indicated that natural and high foliage density shrubs were more similar to each other in species composition than either treatment was to low foliage density shrubs (Table 2). Low foliage density shrubs differed from natural and high foliage density shrubs by having higher relative abundances of *P. clemata* (Salticidae) and *Metepeira foxi* Gertsch & Ivie 1936 (Araneidae) and lower relative abundances of *Ph. histrio* (Philodromidae), *E. pepinensis* (Philodromidae), *O. scalaris* (Oxyopidae) and *Dipoena nigra* (Emerton 1882) (Theridiidae).

Family composition also varied with foliage density (Table 1, Fig. 3B). A SIMPER analysis showed that natural and high foliage density shrubs were more similar to each other in family composition than either treatment was to low foliage density shrubs (Table 3). Low foliage density shrubs differed from natural and high foliage density shrubs by having higher relative abundances of jumping spiders (Salticidae) and orb-weavers (Araneidae) and lower relative abundances of Oxyopidae, Philodromidae, and Theridiidae. Dictynids were more abundant on natural foliage density shrubs.

Experimental treatments had no effect on spider guild composition, regardless of classification used (Table 1, Fig. 3C). In general, the distribution of spider guilds was similar across treatments.

DISCUSSION

Habitat structure is cited as an important factor in the distribution and abundance of various organisms (see reviews in Bell et al. 1991). Results presented here demonstrate that spider density and species richness and diversity (H′) are influenced by changes in shrub architecture. High foliage density shrubs supported more spiders and more species than structurally less complex habitats (i.e., low and natural foliage density shrubs). Our results are generally consistent with other studies involving structural influences of vegetation on spiders (Hatley and MacMahon 1980; Greenstone 1984; Brierton et al. 2003). This pattern of greater abundance and diversity on more dense or structurally complex habitats often is attributed to the availability of more microhabitats or as a way to partition resources and reduce interspecific competition (Uetz 1991; Wise 1993).

Variations in spider species and family composition were also observed and were caused by changes in relative abundances, rather than differences in taxonomic composition. For example, although *P. clemata* (Salticidae) was the most frequently captured spider on all shrub types, its relative abundances were higher on low foliage density shrubs. Open substrates may collect a higher proportion of jumping spiders, since dense branching can obstruct their vision and impede their ability to capture prey (Hatley & MacMahon 1980). Since jumping spiders are active hunters that leap onto prey, more compact branching may further interfere with their ability to jump (Stratton et al. 1979). Structurally simple environments also supported relatively more orb-weaving spiders. Wide gaps between shrub branches are considered structurally more suitable for the building of large orb webs than shrubs with more dense architectures (Hatley & MacMahon 1980; Marc & Canard 1997) and may also be associated with larger species of web builders (Hatley & MacMahon 1980).

Table 1.—R^2 and P values from PERMANOVA analysis of spider species, family, and guild composition. For guild composition, values preceding a slash indicate results following the classification proposed by Uetz et al. (1999), whereas values following a slash indicate results when guild assignments followed the classification used for spiders on big sagebrush. PERMANOVA analyses are based on Bray-Curtis dissimilarities.

	Species		Family		Guild	
	R^2	P	R^2	P	R^2	P
Foliage density treatment (FDT)	0.043	0.004	0.045	0.003	0.023 / 0.028	0.245 / 0.176
Prey attractant treatment (PAT)	0.010	0.316	0.010	0.354	0.011 / 0.012	0.317 / 0.264
FDT × PAT	0.013	0.677	0.024	0.163	0.028 / 0.026	0.144 / 0.190

Structurally diverse environments, on the other hand, may be chosen by species that attack their prey within close proximity. For example, although thomisids were largely underrepresented in this study, they are thought to prefer more concealed locations for prey capture (Hatley & MacMahon 1980; Uetz 1991). Space-web builders (Dictynidae and Theridiidae) may also require more complex substrates, since they tend to build three-dimensional webs that occupy small spaces between branches (Stratton et al. 1979; Marc & Canard 1997).

Despite notable differences in spider species and family composition, guild composition did not vary by foliage type. These results contradict previous studies suggesting that habitat structure influences the distribution of spider guilds found on big sagebrush (Hatley & MacMahon 1980; Robinson 1981; Abraham 1983; Wing 1984; Heikkinen & MaeMahon 2004) and elsewhere (Uetz et al. 1999; Brierton et al. 2003). Discrepancies between research findings may have been due to underlying differences in field site characteristics. Previous studies in northern Utah were mostly conducted at sites with elevations more than 200 m below our study area (Hatley & MacMahon 1980; Robinson 1981; Abraham 1983; Wing 1984). Since spider composition is known to vary with elevation (Bowden & Buddle 2010; Cardoso et al. 2011), it is possible that factors associated with elevation, such as temperature or vegetation structure, contributed to changes in relative abundances of species or families across field sites that then translated into major differences in guild structure. For example, Abraham (1983) found a higher proportion of some families (Theridiidae and Thomisidae), but a lower proportion of others (Dictynidae, Oxyopidae, and Salticidae), relative to our study site. Patterns of guild abundance and distribution may also have been influenced by cattle during part of this study, as some spiders are known to be particularly sensitive to livestock grazing and trampling (Warui et al. 2005).

The lack of guild response may also suggest that individual species have specific ecological requirements that cannot always be captured using a guild approach. For spiders, guild membership is usually taxonomically based, since spider hunting strategies are thought to emerge at the family level (Post & Riechert 1977). However, many suggest that these generalizations are not entirely applicable to all species, or at all times, and that guild membership should reflect natural histories, rather than taxonomic relatedness (Hawkins & MacMahon 1989; Uetz et al. 1999). Although the use of guilds in this study revealed little about the relationship between spider hunting strategies and shrub architecture, the concept is still useful for examining competitive interactions and niche relations in ecological studies or when comparing communities that vary in space and time (Hatley & MacMahon 1980; Hawkins & MacMahon 1989).

Table 2.—Summary results of a similarity of percentages (SIMPER) analysis of spider species composition among shrubs of different foliage density treatments (i.e., low, natural, or high). Results indicate average relative abundance, range of contribution (%) to Bray-Curtis dissimilarities and pairwise comparisons of dissimilarities among treatments. Only species that consistently contributed ≥ 5% are shown.

Species	Low	Natural	High	% Contribution
P. clemata	25.98	25.46	18.62	12–15%
P. histrio	11.82	13.22	13.92	11–12%
E. pepinensis	5.62	8.91	13.16	7–9%
M. foxi	9.59	4.30	6.31	7–9%
O. scalaris	5.87	8.91	8.55	7–8%
E. reticulata	6.99	8.36	5.22	7–8%
D. nigra	4.36	5.63	7.01	6–7%

	Low vs. Natural	Low vs. High	Natural vs. High
Average dissimilarity (%)	65.46%	69.29%	57.48%

Table 3.—Summary results of a similarity of percentages (SIMPER) analysis of spider family composition among shrubs of different foliage density treatments (i.e., low, natural, or high). Results indicate average relative abundance, range of contribution (%) to Bray-Curtis dissimilarities and pairwise comparisons of dissimilarities among treatments. Only families that consistently contributed ≥ 5% are shown.

Family	Low	Natural	High	% Contribution
Salticidae	34.02	33.01	29.12	16–20%
Philodromidae	21.19	23.88	28.11	18%
Dictynidae	12.20	13.36	9.03	13–15%
Araneidae	10.16	5.46	6.82	12–13%
Oxyopidae	6.43	10.19	9.84	11–14%
Theridiidae	7.06	7.78	10.56	11–13%
Thomisidae	5.46	3.47	2.38	6–7%
Gnaphosidae	3.49	2.85	4.15	6%

	Low vs. Natural	Low vs. High	Natural vs. High
Average dissimilarity (%)	49.35%	50.96%	38.97%

Results from this study suggest that prey availability is also important in determining spider abundance and species richness. Spiders may have responded to higher prey densities by either increasing prey consumption, thereby influencing rates of survival, development, and/or fecundity, or by simply migrating from areas of low prey availability to areas of high prey availability (Riechert 1974). Positive relationships could also reflect shared microhabitat preferences or physiological constraints (Riechert 1974; Bonte & Mertens 2003; Horváth et al. 2005), especially considering that prey availability was also positively associated with shrub foliage density. Furthermore, because some spiders are known to ignore prey significantly smaller or larger than they themselves are (Nentwig & Wissel 1986), and are capable of assessing nutritional quality of potential prey (Mayntz et al. 2005), it is also possible that true resource availability was never captured and the importance of prey availability was exaggerated. Since our measure of prey availability did not account for actual prey taken by spiders, future studies should incorporate observations of prey consumption to better understand prey importance for spiders.

Our results demonstrate that shrub architecture and prey availability, considered together, are better predictors of spider density and species richness than either variable considered independently. In addition, shrub architecture was a major factor governing spider diversity (H′) and community composition. However, since potential prey densities were also influenced by changes in shrub architecture, the effect of shrub arehitecture on spider communities may instead be operating indirectly via effects on prey availability, rather than directly. While not addressed here, future studies should explicitly evaluate the role of prey availability in mediating the relationship between shrub architecture and spider communities.

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Appendix 1.—List and numbers of taxa other than spiders collected from sagebrush at Hardware Ranch WMA, northern Utah, 2007–2008. Values represent pooled numbers collected from shrubs across all treatment combinations and sampling dates. An asterisk (*) indicates superfamily rank.

Order	Family	Total number collected
Acari		144
Archaeognatha	Machilidae	11
Coleoptera	Buprestidae	4
	Carabidae	26
	Cerambycidae	1
	Chrysomelidae	1649
	Coccinellidae	128
	Curculionidae	19
	Dermestidae	11
	Elateridae	1
	Histeridae	18
	Melyridae	66
	Mordellidae	5
	Searabeidae	1
	Staphylinidae	1
	Tenebrionidae	1
Collembola	Entomobryidae	5
	Sminthuridae	53
Dermaptera	Forficulidae	2
Diptera	Bombyliidae	1
	Cecidomyiidae	14
	Chironomidae	12
	Chloropidae	68
	Culicidae	2
	Phoridae	18
	Pipunculidae	3
	Sarcophagidae	1
	Sciaridae	27
	Simuliidae	10
	Tachinidae	9
	Tephritidae	35
	Ulidiidae	8
Hemiptera	Anthocoridae	4
	Cercopidae	109
	Cicadellidae	3049
	Dictyopharidae	24
	Lygaeidae	42
	Membracidae	59
	Miridae	2967
	Nabidae	253
	Ortheziidae	7
	Pentatomidae	23
	Psyllidae	47
	Reduviidae	11
	Rhopalidae	3
	Scutelleridae	5
	Tingidae	39
Hymenoptera	Braconidae	28
	Chalcidoidea *	201
	Chrysididae	2
	Cynipoidea *	18
	Halictidae	1
	Ichneumonidae	2
	Vespidae	1
Lepidoptera	Lycaenidae	7
	Noctuidae	299
	Nymphalidae	2
	Pterophoridae	1
Mantodea	Mantidae	1
Neuroptera	Chrysopidae	3
	Hemerobiidae	3
	Myrmeleontidae	1
	Raphidiidae	8
Odonata	Coenagrionidae	2
Orthoptera	Acrididae	57
	Rhaphidiphoridae	2
	Tettigoniidae	32
Psocoptera	Liposcelidae	100
	Psocidae	75
Thysanoptera		87
Total		9929

Appendix 2.—List and numbers of spider taxa collected from sagebrush at Hardware Ranch WMA, northern Utah, 2007–2008. Values represent pooled numbers of adult specimens collected from shrubs across all treatment combinations and sampling dates.

Family	Species	Total number collected
Araneidae	*Aculepeira packardi* (Thorell 1875)	1
	Hypsosinga funebris (Keyserling 1892)	1
	Metepeira foxi Gertsch & Ivie 1936	60
Dictynidae	*Dictyna idahoana* Chamberlin & Ivie 1933	6
	Emblyna piratica (Ivie 1947)	57
	Emblyna reticulata (Gertsch & Ivie 1936)	85
Gnaphosidae	*Micaria gertschi* Barrows & Ivie 1942	31
	Unidentified	1
Linyphiidae	*Erigone dentosa* O. P.-Cambridge 1894	9
Lycosidae	*Pardosa utahensis* Chamberlin 1919	7
Mimetidae	*Mimetus aktius* Chamberlin & Ivie 1935	2
Oxyopidae	*Oxyopes scalaris* Hentz 1845	133
Philodromidae	*Ebo pepinensis* Gertsch 1933	157
	Philodromus histrio (Latreille 1819)	161
	Philodromus sp.	3
	Thanatus formicinus (Clerck 1757)	27
	Tibellus oblongus (Walckenaer 1802)	12
Salticidae	*Evarcha hoyi* (Peckham & Peckham 1883)	2
	Habronattus americanus (Keyserling 1885)	42
	Pelegrina clemata (Levi & Levi 1951)	690
	Phidippus johnsonii (Peckham & Peckham 1883)	24
	Sassacus papenhoei Peckham & Peckham 1895	18
	Synageles idahoanus (Gertsch 1934)	55
Theridiidae	*Chrysso pelyx* (Levi 1957)	1
	Dipoena nigra (Emerton 1882)	81
	Theridion petraeum L. Koch 1872	22
	Theridion sp.	7
Thomisidae	*Mecaphesa lepida* (Thorell 1877)	3
	Xysticus cunctator Thorell 1877	1
	Xysticus gulosus Keyserling 1880	2
	Xysticus montanensis Keyserling 1887	43
Total		1744

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Development of prey-specific predatory behavior in a jumping spider (Araneae: Salticidae)

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Abstract. We examined differences in predatory behavior between two age groups (newly hatched spiders vs. spiders over 12 weeks old) of *Yllenus arenarius* Menge 1868 (Araneae: Salticidae). The spiders hunted three prey taxa (leafhoppers, caterpillars and thrips) for which they possess pre-programmed predatory behavior. The aim of the study was to check the influence of age and experience on pre-programmed predatory behavior and predatory success. Age-dependent changes occurred in four aspects of predation: direction of approach, mode of approach, distance of attack and predatory success.

Keywords: Pre-programmed, experience, age

Over the last two decades there has been a rapid increase in the number of studies of spider predatory behavior (reviewed in Nelson & Jackson 2011; Jakob et al. 2011; Whitehouse 2011). The majority of studies have focused on salticids – spiders with exceptionally good eyesight (Harland et al. 1999; Land & Nilsson 2002) and complex, vision-guided behavior (Jackson & Pollard 1996; Harland & Jackson 2004). The studies have revealed unusual cases of versatility (Jackson 1986; Jackson & Hallas 1986; Wilcox & Jackson 1998), highly specialized strategies (Jackson & Pollard 1996; Jackson & Wilcox 1993a; Nelson et al. 2005) and cognitive feats of their miniature brains that, until recently, were thought to be distinctive only for higher vertebrates (Wilcox & Jackson 1998). Although a number of studies have demonstrated the complexity of jumping spider predatory behavior, few studies have examined how age and experience influence this behavior.

The information about age-related aspects of salticid predation is scanty but can be collected from scattered data in the literature. It has been shown that jumping spiders possess pre-programmed predatory techniques that are specific for some prey taxa (Jackson & Wilcox 1990; Nelson et al. 2005), predatory performance of inexperienced spiders in their initial interactions with prey is less effective than that of the adults (Forster 1977; Edwards & Jackson 1994) and it improves within a few days from emergence, which results in a significant increase in their hunting success (Forster 1977; Edwards & Jackson 1993, 1994). Recently Nelson et al. (2005) documented that predatory techniques may change with age and size of juvenile spiders. Furthermore, it is known that jumping spiders use various pre-programmed cues in prey discrimination (Harland & Jackson 2000; Nelson et al. 2005; Cross & Jackson 2010); they can quickly learn new cues (Jackson & Wilcox 1993b) and form search-images even after one encounter with their prey (Jackson & Li 2004).

We attempted to address the question of the scale of changes in pre-programmed predatory behavior by investigating hunting techniques of *Yllenus arenarius* Menge 1868 (Araneae: Salticidae), a long-lived jumping spider. The spider has a lifespan reaching up to 770 days, the longest life-cycle reported for salticids. Spiders from one cohort are present in the field for up to three spring seasons, which provides an unusually long time for accumulation of relevant experience (Bartos 2005). The spider inhabits open sandy areas of the Central and Eastern Palearctie (Logunov & Marusik 2003), and it is one of the major invertebrate predators in this habitat (Bartos 2011). *Y. arenarius* is a euryphagous predator with a documented lifetime diet (Bartos 2004, 2011) and predatory techniques (Bartos 2002, 2007, 2008). Hunting prey that is likely to escape (leafhoppers, flies and grasshoppers), the spiders hide their movement from their prey by stalking and movement masking. They approach along the shortest path and attack from a long distance. Hunting prey that is unlikely to escape (caterpillars), the spiders approach quickly, maneuver to approach their prey head on (frontal approach), attack the prey from a short distance and often jump away temporarily leaving the prey. Spiders hunt thrips in an intermediate way (Bartos 2002, 2007). All the aspects are present in early predatory interactions (Bartos 2008).

In the present study we focused on age-dependent changes of pre-programmed predatory behavior with prey likely or unlikely to escape. We addressed two specific questions. First, do any aspects of predatory behavior change with age (and what is the scale of such changes)? And second, are the changes accompanied by an increase in predatory success?

METHODS

Predators.—We collected *Yllenus arenarius* from a dune in Central Poland (Kwilno, 51°59′N, 19°30′E). We used two age groups: a) newly hatched spiders (not older than one week) and b) spiders over 12 weeks old (juveniles 13–16 weeks old and adults 59–120 weeks old). We estimated spider ages on the basis of spiders' phenology, size, and maturity according to a previously developed method (Bartos 2005). We obtained young spiders directly from the field soon after they had emerged from their sub-sand nests. We estimated the date of emergence based on prior studies (Bartos 2005) and started to search for spiders three weeks before the expected date of emergence. We searched daily for 4 h between 09:00 h and 13:00 h, which enabled us to check about a quarter of the whole area inhabited by the population of *Y. arenarius* studied. We divided the dune into quadrats (10 ×10 m) and searched them randomly (each quadrat for 20 min). We intensified the search (to 6 h, starting at 09:00) after we found the first individual from the new cohort. We collected spiders for seven days from the day we found the first spider. This method did not completely exclude the possibility that newly hatched spiders could have had prior experience with prey;

however, such a possibility was low for the following reasons: a) the prey items used in the tests were rare on the sand surface in the period of the study, especially in the bare areas of the dune where we collected young spiders (only three out of over 200 juveniles were found with prey) (M. Bartos unpubl. observ.); b) for a period of about two days after hatching young spiders from the same nest remained close to each other, which signifies that the tendency to disperse was limited. Some authors have suggested that predatory behavior is suppressed in this period (Forster 1977; Edwards & Jackson 1994), which also seems likely for *Y. arenarius*.

In order to reduce the influence of laboratory conditions on the spiders' behavior (Carducci & Jakob 2000) we carried out the experiments on the same day or the day after we collected the spiders. Before the experiments we kept spiders individually in glass containers (10 cm height, 10 cm by 10 cm width) with a layer of dune sand on the bottom. For the experiments we chose each spider randomly and used it only once in the tests.

Voucher specimens of *Y. arenarius* have been deposited in the Arachnological Collection of the Department of Zoology, University of Podlasie, Siedlce, Poland.

Prey.—We gave spiders three prey taxa with different abilities to escape (Table 1): leafhoppers (Hemiptera; prey likely to escape), caterpillars (larval Lepidoptera; prey unlikely to escape), and thrips (Thysanoptera; prey with intermediate ability to escape). We observed prey-specific hunting behavior for catching all of these prey types in earlier studies with *Y. arenarius* (Bartos 2007, 2008). Leafhoppers and caterpillars occur in the natural diet of all age groups of *Y. arenarius* (Bartos 2011). Thrips occur in the diet of spiders up to their 16th week of life. Older spiders do not accept thrips due to the small size of these insects (Bartos 2011); therefore, we gave thrips only to newly hatched and 13–16-week-old spiders. We collected leafhoppers and thrips in the field by sweep-netting dune grass on the day of the experiment or the day before and held them individually in the laboratory. In order to reduce mortality of the prey, we stored insects in a refrigerator at 5°C and took them out 15 min before the experiment started. We obtained caterpillars from a laboratory culture. We measured the body length of prey items with a stereomicroscope and a measuring ocular. Each prey item offered to a spider was within the size rage of ± 20% of the spider's body length. We chose each prey randomly for the experiments.

Experimental apparatus and protocol.—We carried out experiments within a white cardboard arena (15 cm height by 20 cm diameter) with a 1 cm-thick sand layer on the bottom and a millimeter scale placed on the sand. All the experiments took place between 09:00 and 16:00 (laboratory light regime, 12L:12D, lights on at 08:00). Lighting was from a 100W PILA incandescent bulb positioned 0.5 m above the arena and by fluorescent tube ceiling lights 2 m above the arena. First we dropped a spider into the arena and after one min we dropped a prey 8 cm from the spider. We positioned the prey approximately 30° to the left or right of the optical axis of the main eyes to allow the experimenter to record the moment when the predator oriented toward the prey. We left the prey with the spider for 15 min and recorded the interaction with a camera placed above the arena. We released all spiders in the field after the experiments.

Data analysis.—We recorded hunting success in each encounter and analyzed all prey-specific behaviors described in Bartos (2007, 2008). We measured the distance of attack in Corel Draw 9.0 with a millimeter scale recorded together with the hunting sequence. In order to standardize the distance of attack on spider size we also measured the spiders' abdomen length with a stereomicroscope and a measuring ocular. In the analysis we used the relative distance of attack (distance of attack divided by the spider's abdomen length), which allowed comparison between spiders of different sizes. A linear relationship between the distance of attack and abdomen length ($r = 0.70$, $df = 222$, $P = 0.001$) enabled such standardization (Bartos 2002).

All statistical procedures followed those described by Zar (1984), with statistical tests carried out in Statistica 9.0.

RESULTS

Frequency of prey-specific behaviors.—Age-related differences occurred with each type of prey, but only in two types of behavior: stalk (with leafhoppers) and frontal approach (with caterpillars and thrips) (Fig. 1). Newly hatched spiders stalked their prey less frequently than those more than 12 weeks old ($\chi^2 = 4.11$, $df = 1$, $P < 0.05$). A similar pattern occurred in the direction of attack on caterpillars and thrips. Frontal approach was less frequent in newly hatched spiders than in spiders over 12 weeks old that were hunting caterpillars ($\chi^2 = 6.53$, $df = 1$, $P < 0.02$). Newly hatched spiders hunting thrips performed the frontal approach less frequently than spiders over 12 weeks old ($\chi^2 = 9.29$, $df = 1$, $P < 0.05$). The frequency of other behaviors did not change with age.

Relative distance of attack.—Newly hatched spiders attacked their prey from longer relative distances than did spiders over 12 weeks old (Fig. 2). Differences occurred in the relative distance of attack on leafhoppers ($Z = 5.70$, $P < 0.0001$), caterpillars ($Z = 2.68$, $P < 0.008$), and thrips ($Z = 5.51$, $P < 0.0001$).

Predatory success.—Newly hatched spiders hunting leafhoppers had lower predatory success (66%, $n = 29$) than spiders over 12 weeks old (85%, $n = 93$) ($\chi^2 = 5.28$, $df = 1$, $P < 0.03$). Predatory success in hunting caterpillars and thrips

Table 1.—Prey taxa used in the experiments.

Prey taxon	Order, family	Ability to escape	Body length (mm)
Psammotettix sp.	Homoptera, Cicadellidae	High	2–5
Cryptothrips nigripes	Thysanoptera, Phlaeothripidae	Intermediate	1–2
Thrips trehernei	Thysanoptera, Thripidae	Intermediate	1
Chirothrips manicatus	Thysanoptera, Thripidae	Intermediate	1
Pyralis farinalis	Lepidoptera, Pyralidae	Low	2–8
Autographa gamma	Lepidoptera, Noctuidae	Low	2–8

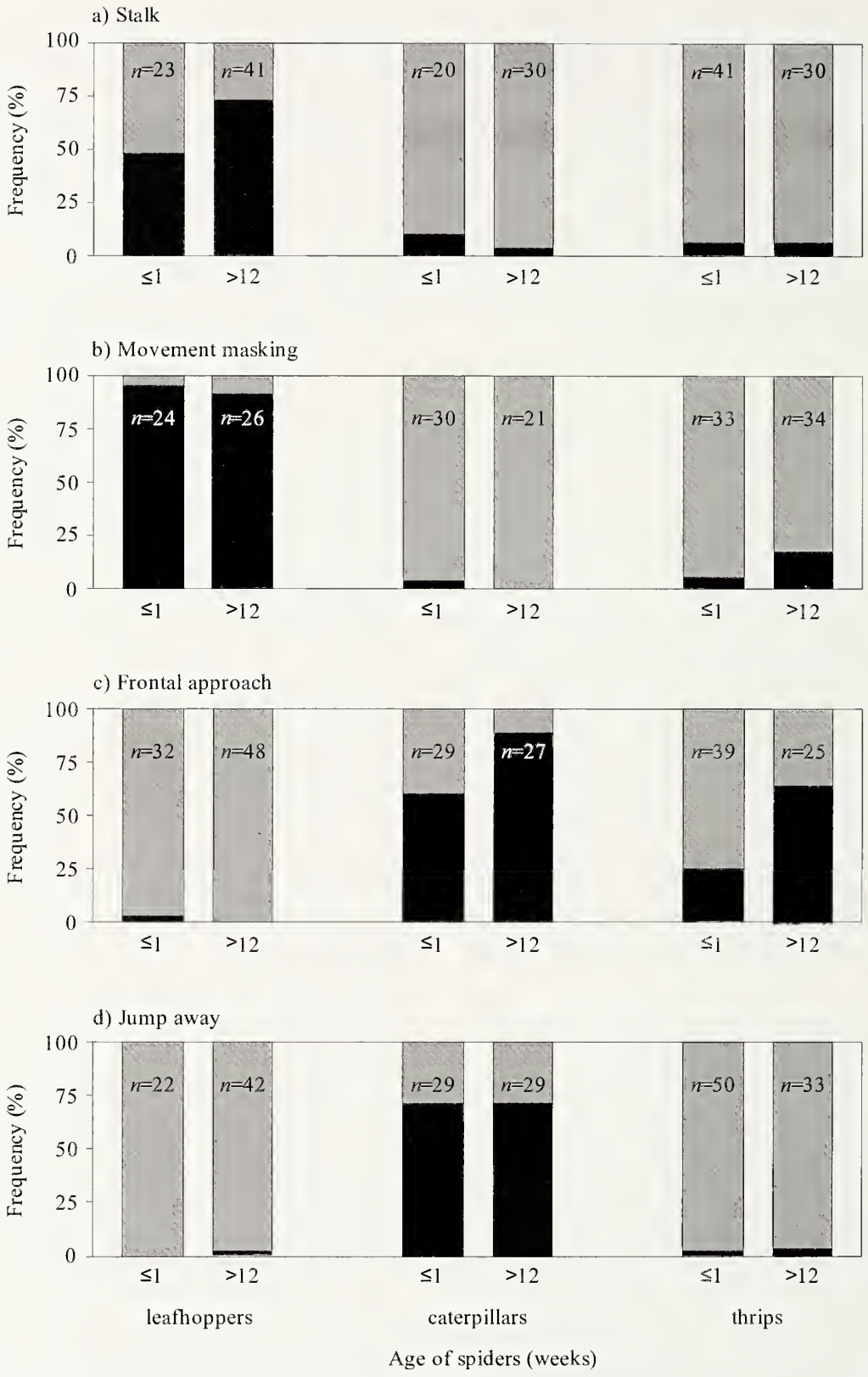

Figure 1.—Relative frequency of four prey-specific behaviors: a) stalk, b) movement masking, c) frontal approach, and d) jump away, in predatory interactions of spiders from two age groups of *Yllenus arenarius* (newly hatched spiders and spiders over 12 weeks old) with three prey taxa (leafhoppers, caterpillars, thrips).

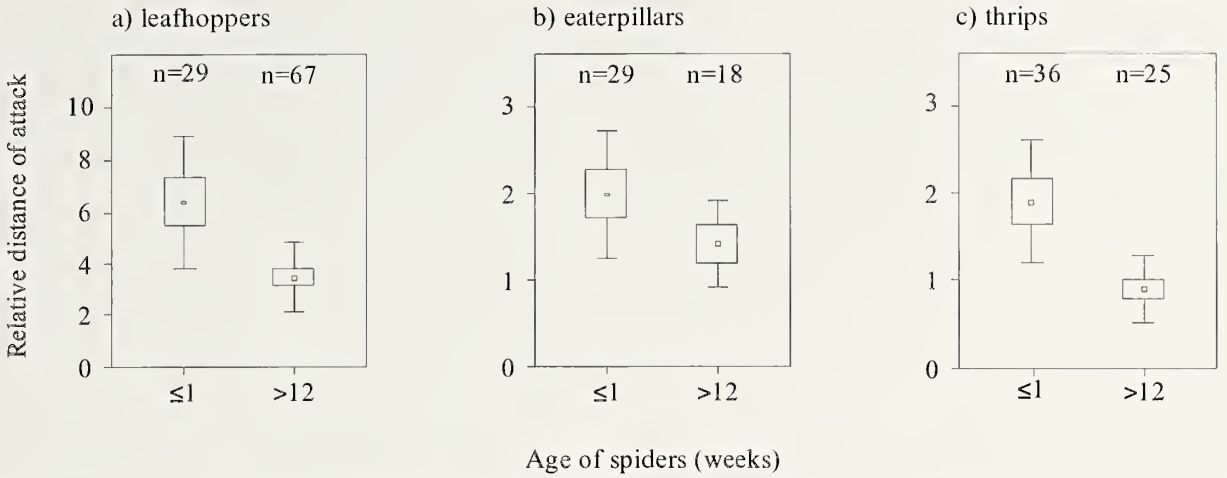

Figure 2.—Relative distances of attack of spiders from two age groups of *Yllenus arenarius* (newly hatched spiders and spiders over 12 weeks old) in interactions with three prey taxa: a) leafhoppers, b) caterpillars and c) thrips. Central point: mean; box: ± 1.96 SE; whiskers: ± SD.

was always 100%, because both groups were unable to escape on the ground. In two out of 84 encounters with thrips, they were captured by the spiders after the insects had risen up into the air.

DISCUSSION

The first noticeable pattern is that inexperienced spiders very frequently used adequate techniques while hunting different kinds of prey. The frequency of adequate prey-speeific behaviors in newly hatched spiders ranged from 48% in stalk and up to 96% in movement masking (both in hunting leafhoppers). The other such behaviors were frontal approach, which oecurred in 59%, and jump away, which occurred in 72% (while hunting caterpillars).

The only prey-specific behavior (out of those examined in the study) (Bartos 2008) that was not eommon in the group of newly hatched spiders was frontal approach in hunting thrips. Only 25% of newly hatched spiders performed it. The situation might have been caused by the relatively high velocity of thrips in comparison to the velocity of newly hatched spiders following them. The thrips were very active, and newly hatched spiders were often unable to walk around running thrips and approach them head on. Thrips may also have features of prey unlikely to escape (elongated body and worm-like movements), and leafhoppers may share features of prey likely to escape (wings and efficiently working legs) (M. Bartos unpubl.). It is possible that the ability to recognize thrips and to choose the proper predatory technique develops with experience, a behavioral modification that would explain why the frequency of performing the adequate behavior (frontal approach) by spiders over 12 weeks old was more than twice as high.

As a result of the initial rather high frequencies of the analyzed behaviors, the scale of the changes occurring later in life could not be large. The changes in the frequencies of those behaviors can be described as refinement rather than dramatic improvement. We saw two kinds of behavioral changes in spiders over 12 weeks old. First, the frequencies of two behaviors specific for hunting particular kinds of prey changed: while stalking, more older spiders hunting leafhoppers approached more slowly, performing choppy, robot-like gait, and while making a frontal approach, more older spiders hunting caterpillars and thrips approached head on. And second, the distances of attacks on all prey types changed: spiders over 12 weeks old jumped on their prey from half the distance used by newly hatched spiders. One explanation of this change, which occurred with all prey, is that precise pre-programmed setting of the optimal distance of approach to prey may not be possible, but that setting can be tuned with experience in successive trials.

The high frequency of adequate prey-specific behaviors in newly hatched spiders suggests that those behaviors are important elements of the spider's predatory strategy. The behaviors may influence the spiders' predatory success in several ways: by decreasing the risk of early detection and prey escape before the attack (stalk, movement masking, long distance of attack on leafhoppers), by increasing the precision of venom injection and avoidance of prey defense mechanisms (frontal approach), and by decreasing the probability of being noticed during the period of prey handling (and potentially eaten together with the prey) by other predators hunting nearby (jump away) (Bear & Hasson 1997; Bartos 2002).

The development of spider predatory behavior may be influenced by several factors, such as changes in the predator's body size (Fraser 1967; Schoener 1967), development of motor coordination (Yoerg 1994), development of the eyes and optical neuropiles (Babu 1975), experience gained in prey recognition (Herberstein et al. 1998; Skow & Jakob 2005; Cross & Jackson 2010) and experience in prey capture (Bailey 1985; Blois & Cloarec 1985; Heiling & Herberstein 1999; Morse 2000). The majority of factors listed above seem to play minor roles in the development of predatory techniques of *Y. arenarius*. The development of motor coordination seems an unlikely factor, as all the behaviors are easily performed by newly hatched spiders (Bartos 2008), and none of the behaviors required from the spiders any exceptional motor coordination. In addition, newly hatched spiders seemed to perform even more physically demanding behaviors than spiders over 12 weeks old (newly hatched spiders hunting each prey type jumped about twice as far as spiders over 12 weeks old). Prey recognition also seems to be of minor importance, because proper prey categorization was very high in the

hunting of newly hatched spiders. These spiders committed very few mistakes, hardly ever using inadequate techniques. They very rarely jumped away while hunting thrips and never jumped away while hunting leafhoppers; they sporadically approached leafhoppers head on and they rarely stalked or masked their movements while hunting caterpillars or thrips. For the same reasons eye development seems an unlikely mechanism to explain the observed changes. It is possible, however, that the longer distance of attack observed in newly hatched spiders resulted from imprecise distance estimation of incompletely developed eyes. The most likely factor influencing predatory techniques of spiders over 12 weeks old seems to be the experience gained in hunting successive prey (Edwards & Jackson 1994). In consequence of such experience the spiders' pre-programmed behavioral patterns may become tuned to the prey they encounter in their natural habitat. Such a refinement seems to occur in all analyzed aspects of behavior and might be sufficient to explain the slight increase in hunting success.

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The effect of leg autotomy on terrestrial and aquatic locomotion in the wolf spider *Pardosa valens* (Araneae: Lycosidae)

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Abstract. Many species use autotomy, the self-amputation of an appendage, as a last-gasp method to escape a predator. Although this behavior can have immediate survival benefits, it can also negatively affect future survival or reproduction. The wolf spider *Pardosa valens* Barnes 1959 occurs along small mountain streams in southeastern Arizona, where it moves both on cobble along the stream and on top of the water's surface. Autotomy of legs is common in this species, and we hypothesized that such leg loss could lead to decreased sprint speed in both terrestrial and aquatic locomotion. We examined burst speed in the laboratory on artificial terrestrial and aquatic racetracks during 2005 (both males and females) and 2006 (females only). In 2005 terrestrial trials, intact spiders were faster than autotomized spiders, but there was no effect of sex on speed. In contrast, 2005 aquatic trials revealed that females ran faster than males, but that autotomy had a negative impact on the speed of females only. Additionally, female spiders generally ran faster on the terrestrial track later in the day than earlier in the day, suggesting that environmental variables such as temperature may have some influence on spider locomotion. Males were less likely to run on water than were females, and ran shorter distances when they did run. Results for females during 2006 also showed a decline in speed with autotomy, and an increase during later trials, although the results were weaker than during 2005, with only the aquatic trials showing a significant difference. These results suggest that leg autotomy in this spider does have a cost, but that the magnitude of this cost depends on aspects of the spider (e.g., sex) and habitat (e.g., substrate and environmental conditions).

Keywords: Sprint speed, performance

Many animals autotomize a body part as a defensive behavior (reviews in Maginnis 2006; Fleming et al. 2007). The body part sacrificed is typically an appendage that can be easily grasped: tails in lizards (Arnold 1988; Bateman & Fleming 2009), salamanders (Brodie 1983), and dormice (Juškaitis 2006); arms in echinoderms (Bingham et al. 2000); caudal lamellae in damselfly larvae (Robinson et al. 1991); and legs or claws in various arthropods (e.g., Carlberg 1994; Juanes & Smith 1995; Foelix 1996; Guffey 1998). Although autotomy may occur during intraspecific agonistic competition (e.g., Dodson & Beck 1993), as a response to toxins (e.g., Eisner & Camazine 1983), or during molting (e.g., Maginnis 2008), most studies suggest it results primarily from interaction with predators (Maginnis 2006; Fleming et al. 2007). Regardless of the cause, autotomy has obvious selective advantages if it allows an individual to survive an encounter that it otherwise might not.

Despite the immediate benefits, loss of the autotomized body part is not without potential future costs. One such cost arises in animals, such as lizards or echinoderms, which can regenerate the lost appendage. Regeneration diverts energy away from other processes and can lead to decreased fecundity or slower overall growth (Maginnis 2006). A second type of cost arises when lack of the body part negatively affects performance of some behavior. Individuals with an autotomized appendage may run more slowly, be more susceptible to predator attacks, be less competitive for mates, have lower prey capture success, or have lower social status than intact individuals (reviewed in Fleming et al. 2007).

A number of spiders will autotomize a leg under life-threatening circumstances (Roth & Roth 1984; Foelix 1996). For wolf spiders (family Lycosidae), this behavior appears to be a moderately successful way to survive being grasped by a predator (Klawinski & Formanowicz 1994; Punzo 1997). It is also relatively common, with previous studies indicating that 8–32% of individual wolf spiders collected from natural populations were missing at least one leg (Brueseke et al. 2001; Apontes & Brown 2005).

Most small-bodied wolf spiders are cursorial foragers that do not build prey-capture webs; instead, they rely on short sprint bursts both to obtain prey and avoid capture by predators. Thus, the loss of a leg might be costly to wolf spiders if it reduces running speed. Under some conditions, this cost might be slight or nonexistent. For example, leg autotomy did not affect normal locomotion of female *Pardosa milvina* (Hentz 1844) (Brueseke et al. 2001), nor did it affect prey capture success in laboratory settings for this species (Brueseke et al. 2001), *Schizocosa ocreata* (Hentz 1844) (Amaya et al. 2001; Wrinn & Uetz 2008) or *Trochosa terricola* Thorell 1856 (Amaya et al. 2001). However, the cost of leg loss might be greater in situations in which spiders run at or near their maximum speed, as when confronted by a predator. Decreased maximum running speed following autotomy has been found for male and female *Pirata sedentarius* Montgomery 1904 (Apontes & Brown 2005) and for female *S. ocreata* and *T. terricola* (Amaya et al. 2001), suggesting that these spiders may pay a price for autotomizing a leg.

In this study, we examined the effects of leg autotomy on terrestrial and aquatic burst speed in the wolf spider *Pardosa valens* Barnes 1959. This spider occurs in the cobble zone of small mountain streams of southeastern Arizona, USA, moving easily both on land and on top of the water's surface. Based on prior research, we expected that leg loss would negatively affect terrestrial speed, but no studies of which we are aware have examined the effect of leg autotomy on aquatic speed. We therefore determined maximum sprint speeds of

each sex on both surfaees in order to address two primary questions: Does leg autotomy affect sprint speed on either substrate? And, do males and females differ in sprint speed on either substrate?

METHODS

Collection of study animals.—*Pardosa valens* is a small (30–140 mg) wolf spider found from Arizona and New Mexico south into central Mexico (Barnes 1959). As in many spiders, the sexes are dimorphic in size with females larger than males. At our study site in the Chiricahua Mountains of southeastern Arizona, *P. valens* occurs at an estimated density of 500–700 individuals per 50 m stretch of stream, and leg autotomy is common (25–30% of females and 40–45% of males missing at least one leg; C. Brown, D. Formanowicz & C. Amaya, unpublished data).

We collected adult spiders with all legs intact on 15 June 2005 (24 females, 35 males) and 16 July 2006 (25 females) from the cobble zone along Cave Creek, ~0.7 km NW of the Southwestern Research Station (SWRS), Cochise Co., Arizona (31°52′59.5″N, 109°12′20″W, altitude = 1620 m). We returned all spiders to the laboratory at SWRS and weighed them using an analytical balance (to the nearest 1 mg). Spiders were then housed in the laboratory in 15-ml centrifuge tubes stoppered with a wetted cotton ball and laid horizontally. We did not offer the spiders prey either before or during the subsequent experiments, a period of starvation (4 d) which is likely well within the range experienced in the field (e.g., Nyffeler & Breene 1990). The laboratory was exposed to an ambient light and temperature regime.

Experimental protocol.—On the day following collection, we conducted terrestrial and aquatic sprint speed trials for all intact *P. valens*. For each sex, we randomly assigned half of the individuals to be tested first on the terrestrial track and then on the aquatic track; substrate order was reversed for the remaining individuals. We allowed each spider a minimum recovery time of 6 h between trials.

We conducted terrestrial running trials along a 1 m racetrack. This consisted of a square piece of acrylic tubing (2.5 cm width) glued to a plywood board and marked off in 25 cm intervals. At one end of the race track, we glued a small section of tubing which could be blocked at either end by removable pieces of index card ("gates"); this served as the holding chamber for a spider before a trial began. Spiders were placed individually into this holding chamber and given 15 min to acclimate. One of us (CAB) then removed the gates and, using a square piece of cardboard glued to the end of a glass rod, tapped the spider's rear legs to initiate running. As the spider ran, the glass rod was pushed down the track to prevent the spider from turning around and retreating, and the rear legs were tapped again if the spider stopped. The rod was never used to push the spider. A second person (DRF) measured the time required to run each of the four 25-cm segments using a hand-held stopwatch. A single trial was done for each spider.

The aquatic track was constructed using 10-cm diameter polyvinylchloride (PVC) sewer pipe. A section 1 m in length was halved lengthwise, and a PVC cap was cemented to each end. We then sealed the joint between the pipe and cap using silicone rubber. We marked the floor of the pipe in 10-cm segments, beginning at the contact point with one cap edge. Tap water was added to a depth of ~3 cm; preliminary trials indicated that spiders ran normally using this source of water. One of us (DRF) began a trial by holding a spider's centrifuge tube at one end of the track, with the open end of the tube ~1 cm above the water's surface, and gently tapping on it to induce the spider to exit. Spiders that ran down the track immediately were not prodded. If the spider did not run, or if it moved backward under the cap, it was gently prodded using the tip of the centrifuge tube or a pair of forceps until it ran in the correct direction or until we were sure it would not run at all (see Results). As in the terrestrial trials, a single run was done for each spider. All aquatic trials were videotaped with a digital camcorder (Sony Handycam model DCR-PC1000). From these recordings, one of us (CAB) recorded the maximum distance traveled by a spider without stopping, and, using a stopwatch, the time required to run this distance.

Following completion of trials using intact spiders, we induced each individual to autotomize a haphazardly-selected leg by grasping the femur of the leg with fine forceps; the leg then autotomized at the coxa-trochanter joint as the spider pulled away (Foelix 1996). This caused a small loss of hemolymph, but the break rapidly sealed and the spider was otherwise unaffected. We then repeated the terrestrial and aquatic running trials two days after legs were autotomized, using the same order of substrate use for each spider as used in the intact trials. Using this experimental procedure, we assume that any physical or psychological stress from autotomy had abated after two days, so that any differences observed in locomotion were due to autotomy alone rather than the stress of the procedure; however, we recognize that this is an untested assumption in our design. After completion of all running trials, spiders were returned to Cave Creek.

Statistical analysis.—For the terrestrial trials, we calculated speed in cm/s by dividing 25 cm by each interval time. For each spider, we used the maximum speed over a single 25 cm interval in our analyses (using other measures, such as speed over the entire meter or mean interval speed, gave qualitatively similar results). For the aquatic trials, we calculated speed in cm/s by dividing the maximum distance run by the time required to run this distance. In all analyses, sprint speed was natural-log transformed to reduce variance heterogeneity.

We analyzed the 2005 sprint speed data using two-factor repeated measures ANOVAs (RM-ANOVA), with sex (male or female) and trial order (aquatic first or terrestrial first) as fixed main effects, and leg loss (all legs intact or with one leg autotomized) as the repeated measure. For the 2006 sprint speed data, where we had only females, we performed one-factor RM-ANOVAs, with trial order as the main effect and leg loss as the repeated measure. Separate RM-ANOVAs were conducted for the aquatic trials and the terrestrial trials in both years. We included trial order as a main effect, because environmental conditions likely varied between the times when trials were conducted. For example, temperatures in the laboratory were higher in the afternoon or early evening trials (30–31.5°C) than they were in the morning trials (25–27.5°C), and temperature is known to affect sprint speed in ectotherms (e.g., Lailvaux 2007).

We also used a two-factor RM-ANOVA to analyze the distance run (in cm) during aquatic trials in 2005; again, main effects were sex and trial order, and leg loss was the repeated

Table 1.—Terrestrial sprint speed in the wolf spider *Pardosa valens* across two years; ANOVA results showing the effects of sex (male or female), trial time (morning or afternoon/evening), and leg status (all legs intact or one leg autotomized). Females were only measured in 2006, and thus sex was not included in the analysis. For 2005, degrees of freedom (*df*) = 1, 45 for all tests. For 2006, *df* = 1, 20 for all tests.

Year	Factor	*F*	*P*
2005	Sex (S)	0.04	0.84
	Trial Time (T)	11.98	0.001
	Leg Status (L)	13.36	0.001
	S × T	5.56	0.023
	S × L	0.19	0.66
	T × L	0.32	0.58
	S × T × L	0.07	0.79
2006	Trial Time	2.51	0.13
	Leg Status	0.87	0.36
	T × L	0.41	0.53

measure. Propensity to run in the 2005 aquatic trials was analyzed using log-linear models, with sex, trial time, and leg status (intact/autotomized) as main effects.

Finally, we compared female sprint speeds between the two years using two-factor ANOVAs, with year and trial order as fixed main effects. All analyses were performed using Statistica version 4.5 (StatSoft 1993), with $\alpha = 0.05$.

RESULTS

Of the 35 males collected in 2005, 10 would not run in the aquatic trials when intact and were excluded from further analyses. Nine males completed the intact aquatic trials but would not run on water following autotomy; these we included in the terrestrial data analysis but removed from the aquatic data analyses. In addition, three females collected during 2006 laid eggs following capture and were excluded from analyses. Females (90.8 ± 22.2 mg, $\bar{x}$ ± SD) collected in 2005 were significantly heavier than males (44.5 ± 7.4 mg; $F_{1,50} = 144.0$, $P < 0.0001$) collected that year and were also significantly heavier than females collected in 2006 (64.9 ± 19.0 mg; $F_{1,48} = 20.5$, $P < 0.0001$). Despite these differences, including log-transformed mass as a covariate did not qualitatively change any of the results in the following analyses; we therefore do not report results using mass-adjusted speeds.

In the 2005 terrestrial trials, spiders were significantly faster when intact than when missing a leg, and spiders running later in the day were significantly faster than those running earlier in the day (Table 1, Fig. 1A). Spider sex did not affect terrestrial sprint speed, and no interactions were significant.

In the 2005 aquatic trials, females were significantly faster than males (Table 2, Fig. 1B). Spiders were again significantly faster when intact than following autotomy, but this effect differed between sexes; female sprint speed declined significantly after autotomy, while male speed did not (Table 2, Fig. 1B). Although there was no significant main effect of trial order, there was a significant interaction between trial order and leg loss. Autotomized speeds were similar to intact speeds for spiders that were run in aquatie trials early in the day, but autotomized speeds were substantially lower than intact speeds in trials run later in the day (Table 2, Fig. 1B).

The 2006 trials involving only females exhibited similar trends to the 2005 trials, as sprint speeds decreased following autotomy and increased during trials run later in the day. However, these effects were weaker than in 2005, with only the leg loss treatment in aquatic trials showing statistical significance (Tables 1, 2; Fig. 1A, B). Intact female speeds in

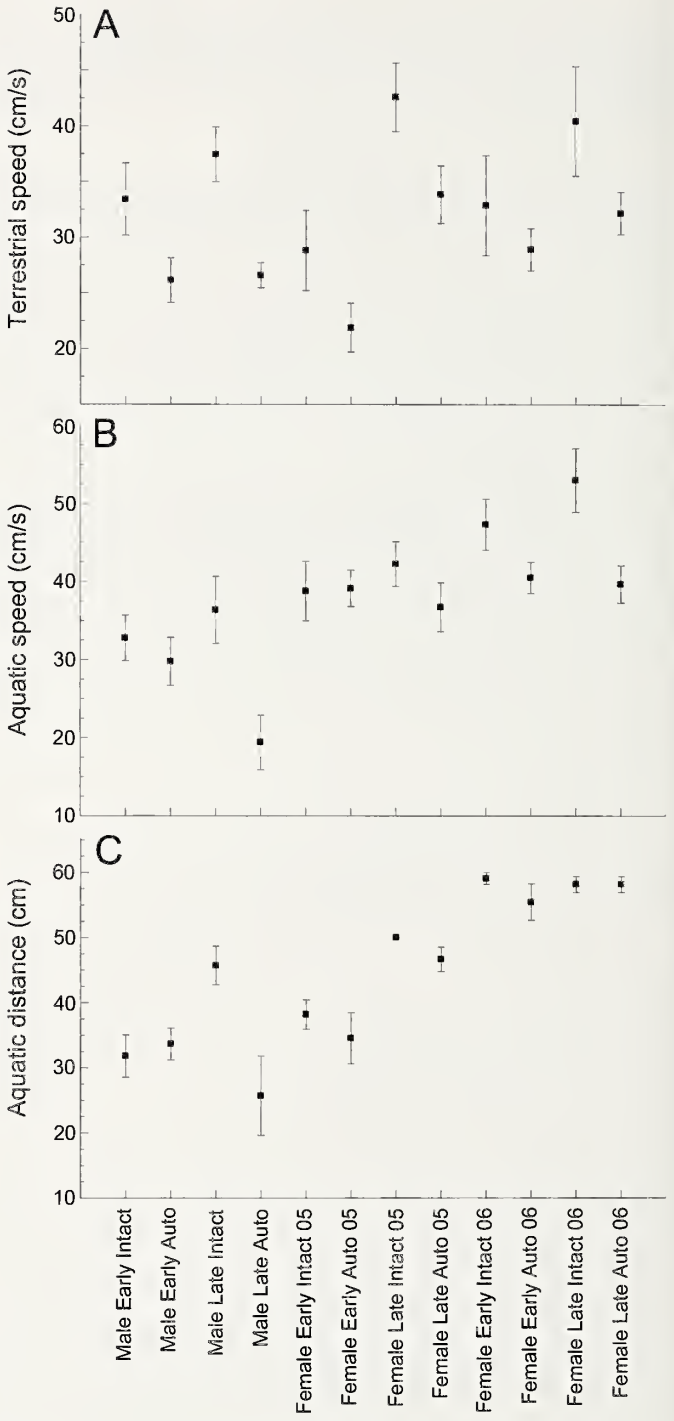

Figure 1.—Mean sprint speeds (±1 SE) on a solid surface (A) and on water (B), and mean distances run on water (C). Early and Late refer to trials run in the morning or the late afternoon/evening, respectively. Intact indicates that all legs were present; Auto indicates that a single leg had been removed. Male data are from 2005 only; females were studied in both 2005 and 2006. In 2005 the distance (C) visible in videos of the aquatic racetrack was 50 cm, while in 2006 the distance visible was 60 cm.

Table 2.—Aquatic sprint speed in the wolf spider *Pardosa valens* across two years; ANOVA results showing the effects of sex (male or female), trial time (morning or afternoon/evening), and leg status (all legs intact or one leg autotomized). Females were only measured in 2006, and thus sex was not included in the analysis. For 2005, degrees of freedom (*df*) = 1, 37 for all tests. For 2006, *df* = 1, 20 for all tests.

Year	Factor	*F*	*P*
2005	Sex (S)	15.16	0.0004
	Trial Time (T)	1.45	0.23
	Leg Status (L)	10.04	0.003
	S × T	1.91	0.17
	S × L	5.59	0.02
	T × L	9.18	0.004
	S × T × L	2.40	0.13
2006	Trial Time	0.38	0.55
	Leg Status	7.94	0.01
	T × L	0.86	0.37

the aquatic trials and autotomized female speeds in the early terrestrial trials were significantly faster during 2006, while other comparisons between 2005 and 2006 were not significantly different (Table 3; Fig. 1A, B).

During aquatic trials, females ran a significantly longer distance before stopping than did males, and spiders ran significantly farther when intact than following autotomy (Table 4, Fig. 1C). Differences between sexes were most pronounced during trials run early in the day. The propensity to run was significantly affected by sex, as male spiders were less likely to run than were females (log-linear analysis, partial association of the sex by run interaction effect: partial χ^2 = 15.4, *df* =1, $P < 0.001$; all other partial associations, $P > 0.15$). In all cases where a spider did not run, it appeared to become trapped in the water's surface film upon exiting the vial.

Spider mass was not correlated with sprint speed on either surface when intact or when missing a leg ($-0.30 < r < 0.24$, all $P > 0.22$). Aquatic and terrestrial sprint speeds were positively correlated in intact males ($r = 0.39$, $P = 0.047$), but were uncorrelated in autotomized males ($r = -0.27$, $P = 0.31$)

Table 3.—Terrestrial (TERR) and aquatic (AQ) sprint speed in females of the wolf spider *Pardosa valens*; ANOVA results showing the effects of year (2005 or 2006) and trial time (morning or afternoon/evening). For terrestrial trials, degrees of freedom (*df*) = 1, 45 for all tests. For aquatic trials, *df* = 1, 43 for all tests. For leg status, INT indicates that all legs were intact, and AUTO indicates that one leg was autotomized.

Substrate, leg status	Factor	*F*	*P*
TERR, INT	Year (Y)	0.24	0.63
	Trial Time (T)	8.40	0.006
	Y × T	0.44	0.51
TERR, AUTO	Year (Y)	3.21	0.08
	Trial Time (T)	13.13	0.001
	Y × T	4.91	0.03
AQ, INT	Year (Y)	5.82	0.02
	Trial Time (T)	2.01	0.16
	Y × T	0.0004	0.98
AQ, AUTO	Year (Y)	0	1.00
	Trial Time (T)	2.19	0.15
	Y × T	1.44	0.24

Table 4.—Distance moved before stopping in the 2005 aquatic trials for the wolf spider *Pardosa valens*; ANOVA results showing the effects of sex (male or female), trial time (morning or afternoon/evening), and leg status (all legs intact or one leg autotomized). For all tests, *df* = 1, 37.

Factor	*F*	*P*
Sex (S)	12.10	0.001
Trial Time (T)	0.01	0.94
Leg Status (L)	18.03	0.0001
S × T	5.30	0.027
S × L	0.13	0.72
T × L	2.95	0.09
S × T × L	17.43	0.0002

and in females during 2005 (intact: $r = 0.12$, $P = 0.56$; autotomized: $r = 0.16$, $P = 0.48$) and 2006 (intact: $r = -0.08$, $P = 0.74$; autotomized: $r = 0.36$, $P = 0.10$).

DISCUSSION

Our results indicate that the loss of a leg negatively affects sprint speed in the wolf spider *Pardosa valens*, but the strength of this effect varies with substrate, sex, and trial order. Looking first at the terrestrial trials, both sexes were significantly slower following leg autotomy during 2005, while in 2006 female speed following autotomy declined but was not significantly different from intact speed. These results generally support the hypothesis that leg loss is costly to terrestrial locomotion, as seen in other wolf spiders (Amaya et al. 2001; Apontes & Brown 2005) and other arthropods (e.g., Carlberg 1994; Guffey 1999; Bateman & Fleming 2005; Fleming & Bateman 2007). However, this cost may be most apparent at faster (sprinting) speeds, such as measured here, rather than slower (walking) speeds (e.g., Brueseke et al. 2001). In spiders, and perhaps other arthropods, decreases in high-speed locomotion following autotomy may result from a change in running behavior. Spider leg movements follow an alternating tetrapod gait at normal walking speeds, with matching legs on either side of the body moving asynchronously, and with their movement resembling an inverted pendulum (Foelix 1996; Moya-Laraño et al. 2008). As movement speed increases, biomechanical traits such as stepping frequency and duty factor change (e.g., Ward & Humphreys 1981; Spagna 2006), which can cause legs to move either more (Ward & Humphreys 1981) or less (Foelix 1996) asynchronously. The loss of a leg could thus make it more difficult for a spider to switch efficiently from low-speed to high-speed mechanics, resulting in decreased speed.

In the aquatic trials, males ran significantly slower following leg autotomy, while female speed decreased significantly only in 2006. This represents the first evidence that leg loss can negatively affect aquatic surface locomotion in a spider, which again may result from a biomechanical change in the way spiders move on the water's surface. Aquatic locomotion occurs in at least six families of spiders, but is most common in the Pisauridae (fishing spiders) and Lycosidae (wolf spiders) (Stratton et al. 2004). Many fishing spiders and wolf spiders, including *P. valens*, use a rowing or galloping motion when on the water's surface, which involves synchronous movements of pairs of legs on either side of the body (Stratton et al. 2004). Autotomy may decrease the efficiency of these motions, for

example by lowering the torque produced by the power stroke on the side of the body missing the leg.

Females were heavier in 2005 than in 2006, and this difference in mass may reflect variation in several traits that could influence how autotomy affects locomotion. First, greater mass could indicate that females collected in 2005 were overall larger in structural size, specifically in leg length, which would have enabled them to attain longer stride lengths and perhaps faster speeds. Although we did not directly measure structural size, we suggest that this interpretation of the size variation is not supported, since it predicts that females would be faster in 2005; instead, we found the opposite to be true. In addition, mass and leg length are not strongly correlated in other wolf spider species (C. A. Brown unpublished data), and neither mass (Apontes & Brown 2005; this study) nor leg length (Apontes & Brown 2005) appears to be strongly correlated with terrestrial sprint speed in wolf spiders (but see Moya-Laraño et al. 2008 for a counter-example in a cobweb spider). Thus, even if females were structurally larger in the 2005 sample, the larger size did not lead to an increase in speed.

An alternative, and perhaps more likely, explanation is that female mass differed between the two years due to differences in body condition that arise from the timing of collection of spiders. Females in 2005 were collected in mid-June, when nearly all were gravid with their first (or, less likely, second) clutch of eggs, while females during 2006 were collected in mid-July, when they were either gravid with a second (or third) clutch or had already oviposited. Since clutch size and mass decline with each successive egg sac produced in wolf spiders (e.g., Brown et al. 2003), 2006 females would be expected to be lighter than those from 2005. On the water's surface, being heavier may be detrimental if this increases the area of contact with the water and thus increases drag. This idea draws support from the fact that lighter 2006 females were significantly faster than heavier 2005 females when all legs were present, and thus suffered a more precipitous decline in speed following autotomy (when speeds were more similar). On land, heavier spiders may be less able to compensate for leg loss than lighter spiders, due to a decreased ability to generate the force necessary to move the spider through the inverted pendulum motion (Moya-Laraño et al. 2008); this is suggested by the greater decrease in speed of autotomized spiders during the early trials in 2006.

Male *P. valens* were significantly slower than females on the water's surface, but not on land. This latter result was surprising, given that male wolf spiders are generally smaller than females in overall body size and leg length, although males may have longer legs for a given body size (e.g., Apontes & Brown 2005). It also contrasts with results for the wolf spider *Pirata sedentarius*, the only other study comparing male and female terrestrial sprint speeds, in which females are significantly faster (Apontes & Brown 2005). Body mass (perhaps reflecting reproductive status) may have affected our results, with heavier (probably gravid) females having terrestrial speeds more similar to males than they would if lighter (post-oviposition). However, we would expect the same pattern to hold during aquatic locomotion, since additional weight appears to lower female speed on water more than on land. Since this was not the case in our study, we remain unsure about the cause of the differences in the relationship of male to female speed between the two substrates.

In addition to running more slowly on water, males were less likely to run than females and, when they did run, ran shorter distances than females before stopping. All cases in which the male did not run exhibited a similar pattern: upon exiting the tube, the male appeared to become wetted and stuck in the surface tension of the water; if prodded, the male would then turn slowly in a circle moving just one or a few legs. In addition, all males that did this in their initial attempt on the aquatic track repeated the behavior when we informally attempted a second time to induce them to run. Based on our field observations, this was an unexpected behavior in *P. valens*. We have observed numerous instances of both male and female spiders moving across the water to avoid capture, and have never seen one beeome entangled in the surface tension as occurred in these trials. Although this may have been due to differences between the tap water used and natural stream water (e.g., presence of planktonic algae in the latter), we think it unlikely that the tap water strongly affected the ability to run, as the majority of males and all females were capable of running on the surface when all legs were intact. A more intriguing possibility is that males differ from each other, and from females, in their ability to prevent capillary adhesion of water, either through differences in cuticular composition or in the density or composition of hairs (Suter et al. 2004). If so, we would predict that males with less ability to prevent adhesion of water should be less likely to be found near water and less likely to attempt aquatic locomotion.

Females ran faster in the later (afternoon/early evening) terrestrial trials than in the earlier (morning) trials, although this time of day effect was significant only in the 2005 trials. For males and for aquatic locomotion, speeds were also generally higher in the later trials, although the differences were small and not significant. Thus, our results suggest that the timing of the trials has some influence on running speed, more so for females and for terrestrial locomotion. Several environmental variables, such as light level, temperature, and relative humidity, may have differed between the two trial periods and thus affected sprint speeds. Temperature in particular is known to affect many physiological processes and behaviors, and room temperatures were on average 4–5°C warmer during the trials performed later in the day. Although little research exists on the influence of temperature on spider locomotion, it would not be surprising for sprint speeds to be positively related to temperature in this group. Locomotory performance is known to increase with temperature in other ectotherms (e.g., reptiles: Lailvaux 2007), and, in spiders, temperature is known to positively influence life history characteristics such as reproductive output and developmental rate (Li and Jackson 1996).

In summary, we find that leg autotomy in the wolf spider *Pardosa valens*, although of obvious benefit for immediate survival in the face of a predatory attack, induces potential costs to future survival in the form of decreased sprint speed both on land and on the water's surface. However, the relative strength of these costs depends on aspects of both the spider (its sex and perhaps its size or reproductive status) and the environment (the substrate, temperature of the air and/or substrate). Since all life stages of *P. valens*, whether intact or

missing legs, are active throughout the day on both surfaces, each of these factors may be an important influence on future survival; future studies which focus on each factor separately should allow us to better understand the relative importance of each.

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Behavioral evidence of pheromonal signaling in desert grassland scorpions *Paruroctonus utahensis*

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Abstract. Behavioral evidence suggests that, in some scorpion species, females deposit a pheromone that attracts mates. To date, however, no pheromone has been identified. The goal of our study was to isolate a pheromone from female desert grassland scorpions, *Paruroctonus utahensis* (Williams, 1968) (Scorpiones:Vaejovidae). We took in situ cuticular washes from female *P. utahensis* in a chloroform-methanol solution; the extract stratified into aqueous and organic layers. In controlled laboratory experiments, most males exposed to female extract (aqueous and organic fractions combined) exhibited pre-courtship behavior, whereas those exposed to the solvent control (2:1 chloroform-methanol) showed no change in behavior. When extract fractions were separately tested, males initiated pre-courtship behavior when exposed to the organic fraction but not when exposed to the aqueous fraction. These data are the first experimental evidenee of a female pheromone in this species and are important early steps toward characterizing any scorpion pheromone.

Keywords: Pheromone, ground-directed chemical signaling, pectines, arachnid, arthropod

The mechanisms mediating ground-directed chemical signaling have received little empirical attention compared with mate tracking via airborne chemical cues. However, some animals, such as snakes, insects and arachnids, detect nonvolatile sex pheromones while moving along the ground. Male red-sided garter snakes follow the trail of female skin rubbings, allowing males to locate females over long distances (LeMaster & Mason 2001). Similarly, male parasitoid wasps (*Aphelinus asychis*) and male minute pirate bugs (*Orius sauteri*) detect and respond to conspecific female deposits on leaves, but they show no response to volatile female odors (Fauvergue et al. 1995; Nakashima & Hirose 1999).

In addition, both chemical and behavioral evidence suggests that spiders follow pheromone trails to find mates. Recent studies have isolated pheromones from spider silks and indicate a wide variety of chemical signals—from nonpolar fatty acids in the agenelid spider *Tegenaria atrica* (C.L. Koch 1843) to small, polar compounds like dimethyl citrate in the wandering spider (Papke et al. 2000; Trabalon et al. 2005; Jerhot et al. 2010). Further, male wandering spiders *Cupiennius salei* (Keyserling 1877) display courtship behavior upon contact with a silk dragline treated with a small concentration of female pheromone (Barth 1993; Tichy et al. 2001).

Several studies suggest chemical cues are important in scorpion mate-tracking and courtship behavior. In a Y-maze test, male desert hairy scorpions *Hadrurus arizonensis* (Ewing 1928) preferred substrate previously walked on by females but showed no preference for substrate previously walked on by males (Melville et al. 2003). Additionally, male dune scorpions *Smeringurus mesaensis* (Stahnke 1957) initiate pre-courtship behavior when they encounter chemical washes of conspecific female epicuticles (Gaffin & Brownell 1992).

Elaborate, sexually dimorphic organs provide further evidence that chemical communication guides male scorpion mate-searching behavior. All scorpions have movable, ground-directed organs called pectines on their mid-ventral abdomen (Cloudsley-Thompson 1955; Foelix & Müller-Vorholt 1983; Hjelle 1990). Pectines are likely important in mate tracking; upon contact with substrate previously walked on by females, pectinal sweeping increases in *S. mesaensis* (Gaffin & Brownell 1992). Additionally, in pectine-ablated *S. mesaensis* males, conspecific female chemical washes did not release pre-courtship behavior (Gaffin & Brownell 2001). In most scorpions, male pectines are longer and contain more chemosensory peg sensilla than do female pectines (Polis & Farley 1979; Swoveland 1978). A sexual dimorphism in peg chemosensitivity might also be present in some scorpion species: single peg stimulations of *P. utahensis* with citric acid evoked a higher response in male sensilla than in female sensilla (Knowlton & Gaffin 2011). Natural selection might have favored larger pectines with selective chemical sensitivity in males if these chemosensory organs help males track female sex pheromones.

In this paper, we present the first steps toward our goal of isolating and characterizing a scorpion sex pheromone. We collected female *Paruroctonus utahensis* (Williams 1968) scorpions during the peak of their mating season and immediately extracted cuticle-associated chemicals. We then exposed conspecific males to the female extract in laboratory experiments. Initial exposures evoked pre-courtship behaviors similar to those described in *S. mesaensis* (Gaffin & Brownell 1992). Subsequent tests on aqueous and organic fractions revealed strong behavioral responses to the organic fraction. Our evidence suggests that the pheromone has low polarity and is highly stable.

METHODS

Research animal.—We collected *P. utahensis* during early September from sand dunes near Monahans, Texas (31°29′N, 102°39′W) at night using UV. We deposited a voucher specimen in the Sam Noble Oklahoma Museum of Natural History in Norman, Oklahoma. The mating season of *P. utahensis* runs from mid-August through early September (Bradley 1988). In many ways, the natural history and surface activity of *P. utahensis* is similar to the well-described ecology of *S. mesaensis* (formerly *P. mesaensis*; Polis & Farley 1979).

Animal care.—The scorpions were kept in the laboratory in 3.8 l glass jars partially filled with sand from the animals' desert environment. We used timed lighting to maintain a 14:10 h L:D cycle; temperature and humidity were kept within

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a consistent range (22°C, 55–60% RH). The scorpions were fed a wax worm once every 2–3 weeks and were provided water twice a week.

Extract preparation.—In these experiments, we tested male response to a female extract. To produce the female extract, we anesthetized 35 adult (at least 200 mg) female scorpions on ice within an hour of collection and then immediately submerged them in a 2:1 chloroform-methanol solvent. After 8 h, the extract was decanted. Two days later in the laboratory, we condensed the extract to a total volume of about 25 ml using a filter flask and a vacuum line. A few ml of water were added as the solution was drying. The resulting solution stratified to an aqueous layer on top and an organic (relatively nonpolar) layer on bottom. The solution was kept in an opaque glass container at room temperature.

Apparatus and trial preparation.—We used infrared cameras (two Defender SP301-C cameras and one Sony CCD-TRV16 camera) to record male scorpion behavior in contained glass arenas (Pyrex© 15 cm diameter, 7.5 cm deep). We divided each arena into four fictive quadrants with small pieces of tape adhered to the arena rim. The bottom of each arena was thinly coated with sand. Infrared light emitted from photodiodes on the cameras was the only light source during these experiments; scorpions are not sensitive to infrared wavelengths (Blass & Gaffin 2008).

We conducted two experiments to determine if the female extract would release pre-courtship behavior in male scorpions. To determine sample size, we conducted an a priori power analysis using data from unpublished research by Gaffin and from pilot studies ($dz = 2.06$; $\alpha = 0.05$; $1-\beta = 0.95$; paired). We concluded that we needed at least six legitimate trials per experiment to be confident in our results (see criteria for legitimacy below); we used extra scorpions in each experiment to ensure we met this minimum. Each trial started between 1–3 h after the beginning of the dark cycle, when scorpions are normally active in the field. The order of treatment exposure to each scorpion was random (as determined by a computer random number generator). The scorpion was placed in the quadrant opposite the test quadrant, initiating the trial. After each trial, we removed the sand, cleaned the arena with 70% ethanol, and placed fresh sand into the arena.

Experiment 1.—In the first experiment, conducted in October and November, we exposed 12 male scorpions to the female extract and to a control solution at separate times. The extract was shaken to create a homogeneous solution before application to the sand on the arena floor; 2:1 chloroform-methanol solvent was used as the control. The trial preparer deposited 100 µl of either the extract or the control solution near the arena wall of the randomly selected test quadrant (by computer random number generator). Scorpions rested at least 2 d between trials.

Experiment 2.—We conducted a second experiment a month after the first experiment ended. Ten of the 12 scorpions used in the first experiment were used for the second experiment. The other two scorpions died between Experiments 1 and 2. For the second experiment, extract fractions were separately tested. The trial preparer deposited 100 µl of either the organic fraction or the aqueous fraction near the arena wall in the randomly selected test quadrant. We placed the scorpion in the quadrant opposite the test quadrant, initiating the trial. Scorpions rested 6 d between trials.

Trial viewing and scoring.—Each trial was recorded (Defender SN501 DVR) and viewed later. Each trial lasted for 45 min after we placed the scorpion in the arena. The trial scorer was blind to the treatment and test quadrant for each trial. Since the test quadrant could be inferred from scorpion placement in the arena, another researcher previewed each trial and recorded when the scorpion began moving; the scorer began viewing the trial 10 s after movement began. The reviewer viewed and scored trials every couple of days. Since the order of treatment exposure was randomized, the reviewer never knew what extract was being tested. The previewer recorded scorpion position relative to the test quadrant after 10 s of movement; analysis of these data suggest that scorpions had no bias toward any quadrant during these initial movements ($X^2 = 0.5$, $df = 2$, $P = 0.779$), keeping the test quadrant unknown to the scorer. Further, no scorpion exhibited courtship behaviors during the first 10 s of movement. The previewer also determined trial legitimacy; in a legitimate trial, the scorpion had to cross the test quadrant at least once.

We based our scoring criteria on pre-courtship behaviors documented in *S. mesaensis* males (Gaffin & Brownell 1992). Before starting experiment 1, we conducted pilot studies on *P. utahensis* to ascertain species-specific differences in pre-courtship behavior. Trials were assigned a score of 1–5 according to the following criteria. 1) No change in behavior. 2) Slight change in behavior, such as creeping (change of normal motion to shorter forward steps and frequent turning) and sudden stops throughout arena. 3) One occurrence of pedipalp-reaching or scrunching (drawing the pedipalps back and toward the body midline), back-up (an abrupt stop of forward motion followed by one or two steps backwards), or sidestepping. 4) Two three-level responses in the same quadrant. 5) Grasping the substrate with the pedipalps, tail wagging, juddering, or three three-level responses in the same quadrant.

Statistical analysis.—In both experiments, only scorpions with two legitimate trials were included in the statistical analysis. All 12 scorpions in Experiment 1 had two legitimate trials, whereas only six out of 10 scorpions in Experiment 2 had two legitimate trials. The final score assigned to each trial was the highest score observed in the test quadrant during that trial. We collected paired, nonparametric data in both experiments and used the Wilcoxon signed-rank test (SPSS software, release 12.0.0) to determine whether treatments within each experiment evoked statistically different behavioral responses.

RESULTS

In these experiments, we exposed *P. utahensis* males to washes from the cuticle of female conspecifics. Of the 22 pairs of trials conducted, 16 (73%) were legitimate. The behavior of male *P. utahensis* changed as they encountered female extracts deposited on the sand substrate of the arena. Back-ups and pedipalp-scrunches were the most common behaviors observed, and males displayed these behaviors to varying degrees. Males that received a score of 5 often continued to perform strong behaviors (sidesteps, back-ups, pedipalp-scrunches) in the test quadrant for several minutes.

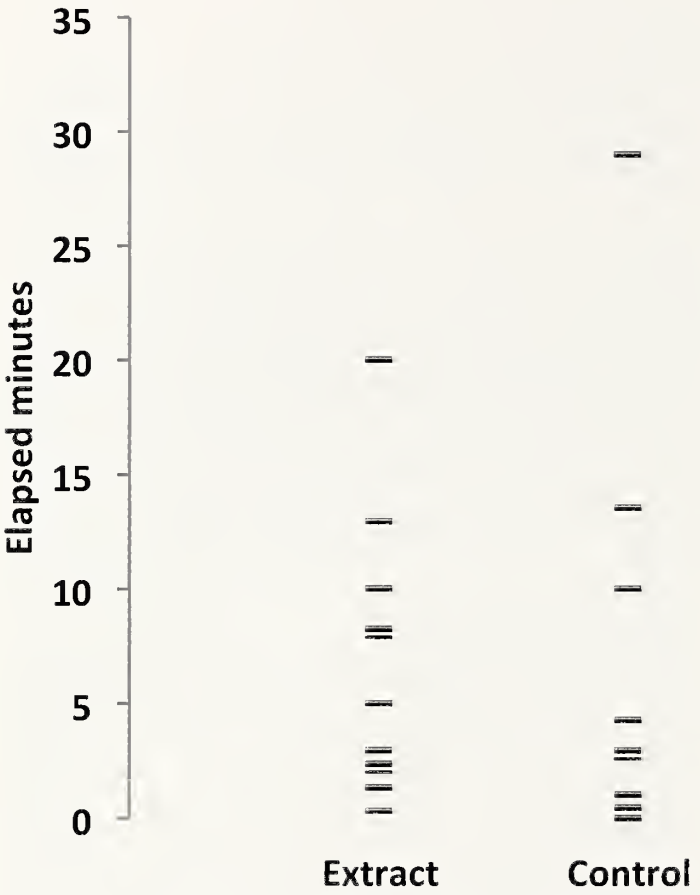

Figure 1.—Distribution of elapsed time before entering treatment quadrant. Shown are durations in minutes for extract and control trials in Experiment 1. Each bar represents the duration for one scorpion.

Others abruptly stopped when walking over the deposited extract, backed up, and then continued walking around the arena (a score of 3). Juddering and tail wagging were each observed in one trial. The strongest response observed was often during a male's first or second crossing of the test quadrant, but in some trials, the male's behavior did not change until he had encountered the deposited extract several times. When males detected no stimulant, they typically walked around the perimeter of the arena and/or attempted to climb the arena walls.

Volatile chemicals from the female extract apparently did not attract males to the treatment quadrant. The average elapsed time before entering the treatment quadrant was 6.7 min in extract trials and 5.9 min in control trials (Fig. 1). In addition, no change in behavior was noted before males entered the test quadrant, providing additional evidence that males are responding to a ground-based chemical.

Experiment 1.—Behavior changed in 10 out of 12 males exposed to homogenized female extracts. The trial scorer also noted changed behavior in two of the 12 control trials, which indicates a low level of experimental error. Males received significantly higher behavioral scores when exposed to female extract than when exposed to solvent control (Fig. 2: $Z = 2.68$, $p < 0.001$, two-tailed).

Experiment 2.—The trial-scorer recorded modified behavior in all six males exposed to the organic fraction of the female

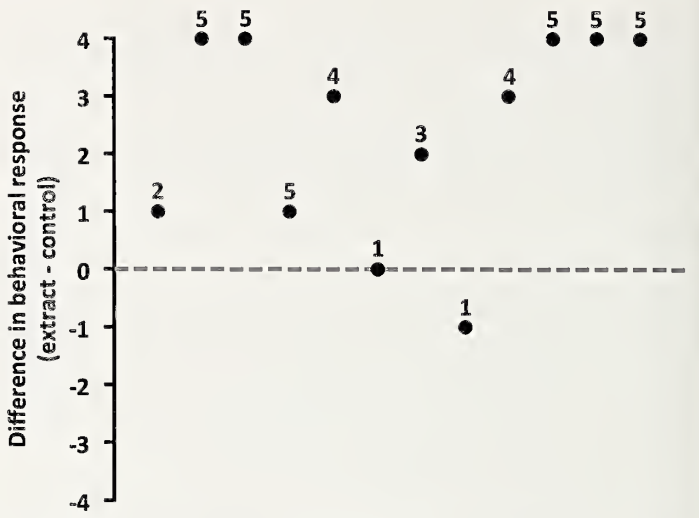

Figure 2.—Comparison of male behavioral responses to homogenized female extract or control fluid of chloroform/methanol. Plotted is each male's difference in behavioral response between trials (extract minus control). Numbers above each point indicate the score of the extract trial. Subtracting the y-axis value from the extract trial value gives the corresponding control trial value.

extract; four males (66%) received the highest behavioral score. No changed behavior was noted as males walked across the aqueous fraction of the female extract. Males received significantly higher behavioral scores in organic fraction trials than in aqueous fraction trials (Fig. 3: $Z = 2.26$, $p = 0.026$, two-tailed).

DISCUSSION

This study represents the first steps toward isolating a scorpion pheromone. Our first experiment revealed that male *P. utahensis* behavior changes when encountering a female chemical extract, which provides the first evidence for substrate-borne chemical signaling in this species. Our second

Figure 3.—Comparison of male behavioral response to fractioned female extracts. Plotted is each male's difference in behavioral response between trials (organic fraction minus aqueous fraction). Numbers above each point indieate the score of the organic trial. Subtracting the y-axis value from the organic trial value gives the corresponding aqueous trial value.

experiment showed that male behavior only changes upon encountering nonpolar chemicals from the female chemical extract. Aqueous chemicals from the female extract released no pre-courtship behavior in any of the males tested.

Males are not attracted to the female extraet at a distance. In none of our studies did males initiate pre-courtship behavior outside of the test quadrant of the arena. In addition, males were not drawn to the test quadrant more quickly in trials with the female extract than in control trials in Experiment 1. We therefore conclude that the chemical (or mixture of chemicals) that males responded to in our studies was not volatile.

Although the pheromonal chemical(s) remain unknown, it is possible that male scorpions are reacting to a lipid in the female cuticle. Nonpolar chemicals, sueh as cuticular lipids, might be more stable in a hot, windy habitat than polar chemicals. In general, longer earbon chains have lower polarity and a higher boiling point than shorter carbon chains. Although we cannot be certain that the female *P. utahensis* chemical signal has long hydrocarbon chains, cuticular extracts from another scorpion, *Hadrurus arizonensis*, show a high proportion of compounds with chains exceeding 18 carbons (Trabalon & Bagnères 2010).

Males responded vigorously to the extract in Experiment 2, four months after initial extraction. In these scorpions' habitat, it is likely that heat, wind, and exposure to sunlight would break down chemicals faster than we observed in our laboratory. Still, winds may blow sand grains covered with female-deposited pheromone across the dunes. If the pheromone remained stable for even a few days, the pheromone could spread long distances from the female. During our collections in the mating season, we observed several males within 20 m of eaeh female, an unusual grouping for *P. utahensis* at this field site. With the dispersing sands, it seems unlikely that males follow a female trail directly to her. Instead, it is more likely that males compare concentrations of female pheromone and move toward increasing concentration.

The stability of the female pheromone is noteworthy and may lead to some interesting follow-up studies. For example, we hope to test male response to fractions within the organic female extract. We have conducted some preliminary trials (two trials each on three different fractions) and did not see the vigorous male responses we observed with the entire organic extract. One scorpion exposed to the most nonpolar fraction stopped in the test quadrant during his first crossing, backed up, and moved forward four times, but no other male responded. The low response rate in these preliminary trials might suggest that male scorpions respond to combinations of female chemicals. However, these observations must be approached with caution, because of the small sample size. In addition, because reproduetion is seasonal, male sensitivity might fluctuate throughout the year, influencing their responses to these fraetions. It is also possible that the active compound or compounds had begun to break down before these trials, which were conducted eight months after the extraction was made.

Since it is likely that males use their pectines to detect female pheromones (Gaffin & Brownell 2001), future studies might focus on pectinal sweeping as animals move across extract-contaminated substrates. For example, Blass and Gaffin (2008) made circular tracks composed of a small Petri dish glued inside a larger Petri dish. In such a setup, the pectines can be filmed from below through a clear Plexiglas stage. Extract fractions could be blotted directly on the Petri dish surfaee or dried onto grains of sand sprinkled in the arena, and the number of pectinal sweeps could be tracked as the animal moved across various stimuli. It might also be possible to use thin-layer liquid chromatography (TLC) to separate the extraet into bands and test the animal directly in a rectangular arena atop the TLC plate. In this case, the monitoring would be from the top, but an angled mirror could provide visual access to pectinal activity.

Identifying scorpion mate-tracking pheromones has several possible implications, from controlling populations of dangerous scorpion speeies (thousands of humans die of scorpion stings annually: Warrell 2007) to understanding the physiology and evolutionary significance of pectines. Given our ability to electrophysiologically record directly from seorpions' pectinal peg sensilla (Knowlton & Gaffin 2010), the identification of pheromone compounds presents a unique opportunity to relate proximate sensory mechanisms with the evolution of a ground-based sexual signaling system.

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SHORT COMMUNICATION

Military base growth in Afghanistan: a threat to scorpion populations?

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Abstract. Coalition military bases in Afghanistan are increasing in area, infrastructure and population due to increased military efforts. From 2004 to 2010, a 40-hectare base in Ghazni, Afghanistan transitioned from a montane shrubland to a small, modern "village." This shift comprised an over 50-fold increase in hardcover and a 20-fold increase in the human population. I searched the base with UV light (n = 43.6 h) for scorpions, especially *Mesobuthus* Vachon 1950, an established, opportunistic scorpion found in Ghazni City, 5 km north. I completed my searches along two traeks (> 5 km total length) and considered all habitats for this scorpion. Anthropogenic microhabitats comprised concrete walls, concrete barriers, gabions or sandbags, each in contact with a dirt or gravel substrate (eight possible); all were thermally appealing (mean = 2.3°C warmer than ambient temperature). Despite the population of *Mesobuthus caucasicus* Nordmann 1840 in Ghazni City and the increase in thermally attractive microhabitats on the base, I found no scorpions. I propose that the rapid anthropogenic change due to base improvements outpaces the capacity of this scorpion to disperse to a new, albeit satisfactory, environment. Here, I report my observations of scorpion diversity and abundance in east-central Afghanistan and the Hindu Kush Mountains, with a focus on the impact of increasing anthropogenic change upon the environment.

Keywords: U.S. Army, anthropogenic change, microhabitats, Asia

Due to wars and an unstable political climate in Afghanistan, our understanding of Afghanistan's flora and fauna lags behind that of other Asian countries (UNEP 2009). For example, Vachon's (1958) comprehensive review of scorpions in Afghanistan is more than 50 years old. His study focused, moreover, on areas near population centers such as the capital region (Kabul) and the Sistan Basin (Kandahar, southwestern Afghanistan). The current U.S.-led War on Terror in Afghanistan is having a two-fold effect—generating both increased infrastructure and increased interest in Afghanistan's environment (e.g., UNEP 2009). Here, I report my observations of scorpion diversity and abundance in east-central Afghanistan and the Hindu Kush Mountains (Fig. 1), with a focus on the effect of increasing human impact upon the environment.

The U.S.-led coalition, along with the Afghan National Army, currently operates over 700 military installations in support of the War on Terror in Afghanistan (Turse 2010). These Forward Operating Bases (FOB) range in size from small, mountaintop outposts occupying a few tens of square meters to grand, city-sized logistical centers, such as Bagram Airfield near Kabul, which is over 1,000 hectares in size. Regardless of their size, where they are located or how long they have been there, these bases are having a significant ecological impact. FOB Ghazni, located in east-central Afghanistan, has been occupied for about eight years and currently houses approximately 1,000 Polish and American soldiers. Based on direct observations, collection of photogrammetric data and personal interviews with local leaders, FOB Ghazni has grown signifieantly in these eight years.

Ghazni Province is located in semi-arid, east-central Afghanistan in the Hindu Kush Alpine Meadow ecoregion (Olsen et al. 2001). The region is mountainous and intersected by fault-controlled valleys with regional elevations ranging from approximately 2,000 to over 4,500 m asl. My observations show that the valley and alpine meadow areas (including FOB Ghazni) are comprised of primarily sandy loam to loamy fine sands with intermittent camel weed (*Cymbopogon schoenanthus* Spreng.), sagebrush (*Artemisia* sp. L.), salt cedar (*Tamarix* sp. L.) and grasses such as *Poa bulbosa* (L.). The most common vertebrates spotted were the yellow ground squirrel (*Spermophilus fulvu,* Lichtenstein 1823), jerboas (Dipodidae de Waldheim 1817), rats/miee (Murinae Illiger 1811) and agamid lizards (*Trapelus* sp. Olivier 1804). Common invertebrates included ants (Formicidae Latreille 1809), cockroaches (Blattodea von Wattenwyl 1882), crickets (Gryllidae Bolívar 1878), spiders (Araneae Clerck 1757), occasional sun spiders (Solifugae Sundevall 1833) and three species of scorpions (this study).

Mesobuthus caucasicus (Nordmann 1840) and *Mesobuthus eupeus* (Koch 1839) (Scorpiones: Buthidae) are medically important species (Chippaux & Goyffon 2008) distributed from the Balkan Peninsula to China (Fet et al. 2000). Adults of these species vary in color from light yellow-brown to brownish and can reach lengths greater than 5 cm. The third scorpion is in the genus *Hottentotta* (Birula 1908) (Buthidae), some of which are also medically important (Chippaux & Goyffon 2008). *Hottentotta* are present throughout Africa, the Arabian Peninsula, and in Asia to Pakistan and India, with adults usually a uniform yellow-brown, sometimes with a darkened mesosoma, ranging 3–13 cm in length (Kovařík 2007).

I used photogrammetric techniques on satellite images from 2006, 2008 and 2010 to collect FOB Ghazni infrastructure changes; data collected for 2004 (inception of the FOB) and earlier were from an interview with the Ghazni Director of Agriculture, Irrigation and Livestock, Haji Sultan Hussein Usman Usmani. Human population estimates are based on the 2001 Taliban-run madrasah population and the January 2010 population.

FOB Ghazni infrastructure and population have grown significantly as a result of troop build-up in Afghanistan (Turse 2010). From its inception in 2004, its paved/gravelled area has increased 54 times from approximately 6,000 m^2 to over 325,000 m^2; its building number has increased from one to over 400 structures; its structure area has increased nearly 34 times from 1,500 m^2 to over 51,000 m^2; and it is estimated that the population has increased from dozens to over 1,000.

I collected ambient environmental data (e.g., temperature, humidity and barometric pressure; Table 1) almost nightly at approximately 20:45 hrs, from 13 May 2009 to 16 September 2009 with a digital weather device. In addition, I collected microhabitat temperatures during each nightly survey with an Extech IR thermometer gun (#42540; range: −50–760°C; 16:1 distance:target resolution).

The weather at FOB Ghazni, at approximately 20:45 hrs, for the period 13 May 2009 to 16 September 2009, averaged 20.3°C, 773 mb

Figure 1.—Map of Afghanistan showing Ghazni Province (outlined) and collection sites (black-filled circles represent *M. eupeus* collection, white-filled circle represents *M. caucasicus* collection and crosshatched circle represents collection of *M. eupeus* and *Hottentotta sp.* (see Table 2)). A generalized map of FOB Ghazni (located at the white-filled circle) is overlaid showing approximate building distribution (gray polygons) and search tracks (dashed and solid lines). A photograph of a collected *M. eupeus* is inset (maximum width of bullet is 5.5 mm). Shaded relief map is modified from NASA.gov, and the regional map is modified from marykellykilims.co.nz.

atmospheric pressure and 27% relative humidity (Table 1). Microhabitat temperatures, also at that time, averaged 2.3°C warmer than ambient evening temperatures (Table 1).

Mr. John Kornman, vector-control specialist on FOB Ghazni, provided me with over two years of vector-based disease control data, which supported Department of Defense (DoD) efforts to minimize pest infestation problems (Goddard 2008). Vector data were compiled by observations, reports and glue-board traps. Although glue boards are the least sensitive of pest traps, they are inexpensive and easy to manage; currently, it is unknown whether scorpions are responsive to these traps (cf. Arthur & Phillips 2003). Approximately 50 to 60 of these were dispersed around the base at any given time and, as an estimate of their usage, approximately 300 were employed between April and July of 2009. Glue boards were checked two to three times weekly. Chemical treatments (e.g., Deltamethrin) were dispensed only in targeted areas – no base-wide broadcasting was allowed (DoD 2008). These treatments were applied to cracks and crevices with a hand duster and were considered methods of last resort.

From September of 2007 to November 2009, vector-control specialists on the base found no scorpions by observation, reporting or trapped on their glue boards. The primary arthropods found during vector-control operations were flies, ants and roaches.

I completed a scorpion survey of FOB Ghazni by searching two tracks (Fig. 1), which were planned based on previous scorpion-collection experience at Forward Operating Bases in Iraq during the summer of 2004 (cf. Stewart 2006a, 2006b, 2007). These searches included all possible microhabitats on the FOB, covering the general ground surface and the eight possible anthropogenic microhabitats (Table 1): two substrata (gravel or dirt) in contact with four vertical surfaces (i.e., HESCO (fortification) barriers, sandbags, concrete barriers or buildings). These tracks were searched, on foot, separately or combined for up to 90 min during Nautical and Astronomical twilights from 13 May 2009 to 16 September 2009 using a multi-bulb UV LED light (~385nm, 4.0mW). In 43.6 hours of UV-light searching of FOB Ghazni, I found no scorpions.

Despite the lack of scorpions on the FOB, Lieutenant Colonel Piotr Lewandowski, Polish Army, Mr. John Kornman, FOB Ghazni vector-control specialist and Mr. Zainallah Bodeen, souk merchant, found seorpions locally and regionally. They collected scorpions at five different off-FOB locations (Table 2) by means of rock-rolling, hand-held UV light and in-house capture. These specimens were brought to the author at the FOB alive and were subsequently killed in 99°C water and preserved in a saturated saline solution (39%) in order to bypass USPS shipping restrictions. Specimens were labelled and shipped to Victor Fet at Marshall University, West Virginia, USA for tentative identification and storage until I returned from Afghanistan. Specimens were washed and stored in 90% isopropyl aleohol and are currently housed at St. Lawrence University.

Seventeen specimens were collected off the FOB (Table 2). Nine specimens of *Mesobuthus eupeus* were located while gathering cobbles from an approximately 2,500-m^2 meadow in the Malistan District, approximately 125 km west of the FOB, inferring a density of one specimen every 250 m^2. One specimen of *M. eupeus* and one specimen of *Hottentotta sp.* were collected in Paktika Province, approximately

Table 1.—Environmental and microhabitat temperatures for FOB Ghazni between May and September 2009. Microhabitat temperatures are at the interfaces of the following elements: "H" is HESCO barrier, "R" is rubble, "D" is dirt, "S" is sandbag, "C" is concrete and "B" is building.

	Environmental/Ambient weather data (n = 53)				Microhabitat temperatures (°C) (n = 53)							
	Time	Temperature (°C)	Barometer (mb)	Humidity (% relative)	H-R	H-D	C-R	C-D	S-R	S-D	B-R	B-D
Mean	2045h	20.3	773	27	23.1	21.8	24.1	23.9	23	21.7	21.9	22.3
Max	2130h	25.6	780	35	28.9	27.7	30.1	31.7	30.9	28.1	25.7	26.8
Min	2000h	5	767	21	11.9	11.2	12.3	9.9	11.7	12.4	13.1	12.6

100 km southeast of the FOB, from a derelict, early 19th-century British fortification made of adobe and rocks where they were found roaming within 1 m of the ground-wall interface. Three specimens of *M. eupeus* were collected in a rubble pile at a small, undeveloped firebase in the Giro District, approximately 50 km south of the FOB. Three *Mesobuthus caucasicus* were collected in and around a home in Ghazni City, 5 km north of the FOB. This collection of 17 scorpions expands the work of Vachon (1958) by extending the known distribution of *M. eupeus, M. caucasicus* and *Hottentotta* sp. into east-central Afghanistan. It, moreover, corroborates Vachon's (1958) altitudinal distribution of *M. eupeus* up to approximately 3,500 m asl. and increases the known altitudinal distribution of *M. caucasicus* in Afghanistan from approximately 1,700 m asl. (Kabul area) to approximately 2,200 m asl. in Ghazni City.

Studies on the anthropotolerance (anthropophily) of scorpion fauna are quite rare (cf., Crucitti et al. 1998). Instead, a scorpion's preferred habitat is usually inferred from observations during collection. Early workers (e.g., Birula 1917) made generalizations about scorpions and human habitats that have mostly been accepted, but not tested. Recent studies, more concerned with their conservation and medical importance than faunal and ecological descriptions, are beginning to highlight scorpion-human interactions (e.g., Vignoli et al. 2005; Mirza & Sanap 2010). Crucitti et al. (1998) suggest that in urban habitats, such as Rome, Italy, various factors enable the maintenance of large scorpion populations in a complex urban epigean system, which generates favorable microclimatic conditions. They found that scorpions preferring limestone microhabitats were selective of urban microhabitats that mimicked a limestone environment (e.g., brick walls in old cellars). McIntyre (1999) suggests that anthropogenic change, as a result of home construction, reduces scorpion-sting occurrence. Basically, the author recommends higher density housing, which minimizes direct contact with undisturbed scorpion habitats.

Because there is no documentation of scorpion populations in this region of Afghanistan, it is my assumption that the FOB's location was suitable for scorpions prior to its establishment. Having demonstrated scorpion populations elsewhere in the region with no evidence of scorpions on FOB Ghazni, suggests that anthropogenic changes may have eradicated any incipient scorpion populations (i.e., *M. eupeus*) on the base.

The primary reason for the lack of scorpiofauna on FOB Ghazni is the use of gravel pavement. Since the base's inception, base personnel have covered areas of high-human use with gravel to reduce dust. As of 2010, gravel pavement covers over 80% of the base to an average depth of 5 cm (over 3,000 m^3). This pavement, however, is unsuitable to scorpions. Humans are changing scorpion habit from a suitable, sandy, sparsely vegetated terrain to an unsuitable, rocky pavement.

Conversely, increases in infrastructure can also allow an increase in microhabitats (e.g., Crucitti et al. 1998). The primary reason for anthropogenic scorpion habitat proliferation is from increases in building number and area. These new structures are creating microhabitats, similar to those found in Ghazni City, that appear to be preferred by the generalist/opportunistic scorpion *Mesobuthus caucasicus*. My data show these microhabitats to be thermally appealing, staying warmer than the ambient air temperature during the high-altitude, cool-summer nights (Table 1).

Combining these two issues, a) incipient eradication and b) increase in microhabitats, can help predict what may occur once this FOB's growth stabilizes. Based on the 16 *Mesobuthus* specimens collected and their chosen habitats, it is probable that the dominant scorpion population during the establishment of the FOB was *Mesobuthus eupeus*, which is thought to be anthropophobic (Birula 1917; Fet 1994). All *Mesobuthus* specimens collected from alpine meadows and derelict structures were *Mesobuthus eupeus* (13 of 16), agreeing with Birula (1917) and Fet (1994) that they do not live well with human activity. The remaining *Mesobuthus* specimens (3 of 16) that were collected in Ghazni City, however, were *Mesobuthus caucasicus*, which are synanthropic animals and thrive with humans (Birula 1917; Fet 1994). The remaining *Hottentotta* specimen appears to be an outlier and of no value in interpreting scorpiofauna associated with anthropogenic change.

It appears that military base growth has had the unintended effect of eradicating a *M. eupeus* population through dust-abatement techniques. Initial increase in human activities at FOB Ghazni may have eradicated a *M. eupeus* population. Based on observations of *M. caucasicus* cohabitating in human dwellings in Ghazni City, stabilization of base growth, with continued human activity, may allow the dispersal and stabilization of pioneer, synanthropic *Mesobuthus caucasicus* into new, thermally appealing anthropogenic microhabitats.

Based on the December 2011 end of the war in Iraq, however, it appears military bases are not long lived. The resultant exodus of coalition troops from Iraqi bases has left them as ghost towns and monuments to a once-thriving war (Hodge 2011). This suggests that military bases in Afghanistan may never "stabilize" into functioning, human habitats. It seems, however, they will become derelict like their

Table 2.—Scorpion collection data.

Collection Date	Location	Grid	Elevations (~m)	#	Species	Sex (♂,♀)	Collector
25 May 2009	Malistan District, Ghazni, AF	33.34N, 67.11E	3,300	9	*Mesobuthus eupeus*	6,3	Lewandowski
29 May 2009	Gomal, Paktika, AF	32.76N, 69.05E	2,000	2	*M. eupeus & Hottentotta sp.*	1,0 & 1,0	Kornman
17 July 2009	Giro District, Ghazni, AF	33.09N, 68.33E	2,100	2	*M. eupeus*	1,1	Kornman
13 August 2009	Giro District, Ghazni, AF	33.09N, 68.33E	2,100	1	*M. eupeus*	0,1	Kornman
2 September 2009	Ghazni District, Ghazni, AF	33.56N, 68.41E	2,200	3	*M. caucasicus*	1,2	Bodeen

Iraqi counterparts, leaving long-term, unsuitable environments for both *M. caucasicus and M. eupeus*. The lack of an active, human environment (i.e., heated) and the impervious pavement may essentially sterilize these FOB areas for the near future. A survey of operating bases in Afghanistan in the years to come may help elucidate whether either scorpion species is able to establish itself in this highly modified terrain.

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SHORT COMMUNICATION

Substrate selection for web-building in *Cyrtophora citricola* (Araneae: Araneidae)

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Abstract. In general, spiders that build long-lasting webs invest a larger amount of silk and consequently a larger amount of energy in their construction than those species that build ephemeral webs. It is expected that spiders that build long-lasting webs choose rigid substrates for web construction to help preserve their investment. I experimentally tested this prediction by confining *Cyrtophora citricola* (Forsskål 1775) (Coddington 1989) spiders (n = 32) in containers provided with firm and unstable substrates for the spiders to construct their webs. This experiment confirms that *C. citricola* strongly prefers firm substrates to which to attach its web when it must choose between a firm and an unstable substrate.

Keywords: Araneids, spiders, orb-web, web-substrate selection

The structure of the habitat can be important for spiders when they select sites to build their webs (Janetos 1986). The substrates selected by spiders for web construction vary across species, and this selection may be especially crucial for spiders that build long-lasting webs. Within araneids, those species with long-lasting webs in general use more silk and invest more time to construct denser webs and are not capable of ingesting and recycling a high percentage of the silk of old webs (Lubin 1986; Townley & Tillinghast 1988; Kawamoto & Japyassú 2008), in contrast to those araneid species that make typical, shorter lasting orbs and are capable of ingesting the silk of their webs. The higher investments of silk, time, and energy by spiders constructing long-lasting webs increase the cost of web relocation (Tanaka 1989), likely imposing strong selection on the behaviors associated with web site choice.

Orb-weaving spiders in the genus *Cyrtophora* construct webs that consist of dense, horizontal orbicular sheets of dry silk with an irregular tangle of dry threads above and below (Wheeler 1926; Lubin 1973). The webs are strong, long lasting, and infrequently rebuilt, and are repaired when damage occurs (Lubin 1973, 1980). Thus the spider's choice of appropriate substrates to which to attach the web is important in order to decrease the probability of damage to the web. This paper experimentally examines the selection of firm vs. unstable substrate as support for the construction of the web by *Cyrtophora citricola* (Forsskål 1775) (Coddington 1989).

I collected 32 adult females of *C. citricola* between April and November of 2009 in the Valle Central of Costa Rica (09°56′N, 34°15′W). I placed each spider in a cardboard frame (27 × 22 × 18 cm: width × height × depth); if a spider did not build its web within four nights, it was released and replaced with another spider.

Spiders do not usually attach silk threads to tightly stretched plastic wrapping material, so I covered the open, broad faces of the frame with this material. I also lined one of the sides of the frame with a sheet of this material and then hung a sheet of paper cut into 12 strips (height 22 cm, width 1.5 cm) in front of this side (Fig. 1). The opposite side was not lined with plastic wrapping material, thus giving the spider sufficient support to construct its web. Six of the strips of each sheet were attached to both ends (giving a firm substrate), and six were attached only to the upper end (giving an unstable substrate), following an alternate order: one strip attached to both ends (odd numbers in Fig. 1) followed by another strip attached to one end (even numbers in Fig. 1). I drew horizontal lines 2 cm apart that divided each strip into 11 sections (A to K, from top to bottom), allowing me to record the heights at which spiders attached threads (Fig. 1).

I gave each spider four nights to build its web (20 of the 32 spiders built a complete web in two nights, 12 more in the next two nights). After a spider wove the spiral, the tendency for an addition of new silk threads decreased drastically unless the web was damaged (G. Barrantes unpubl. data). On the fifth day, I used the coordinates provided by the numbered and lettered strips to record the location of each thread attachment. The tensions on the threads generally pulled the unstable strips out of their vertical alignment, toward the spider's web. After counting the threads, I fed the spider a fly, then cut all threads that were attached to the paper strips. This made the spider rebuild the orb of the web, though part of the scaffolding above the

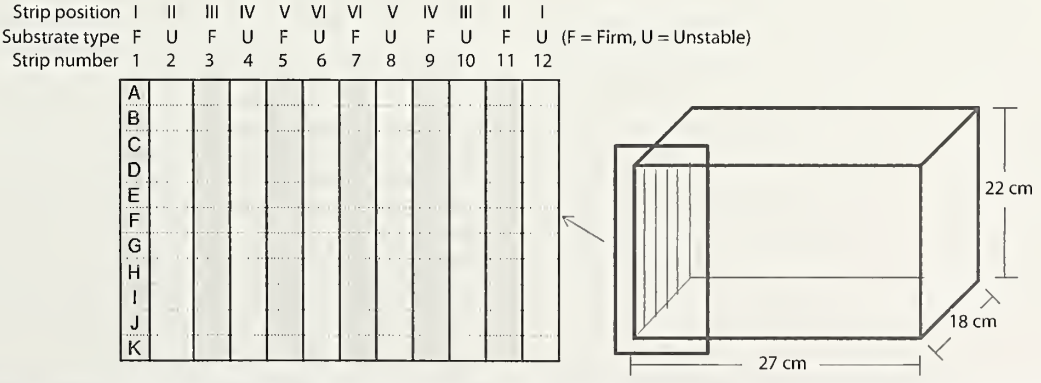

Figure 1.—Stylized drawing of the arrangement of the experimental strips to which spiders attached their threads. F = firm; U = unstable; positions of strips closest to the corners were I, and those nearest the center were VI.

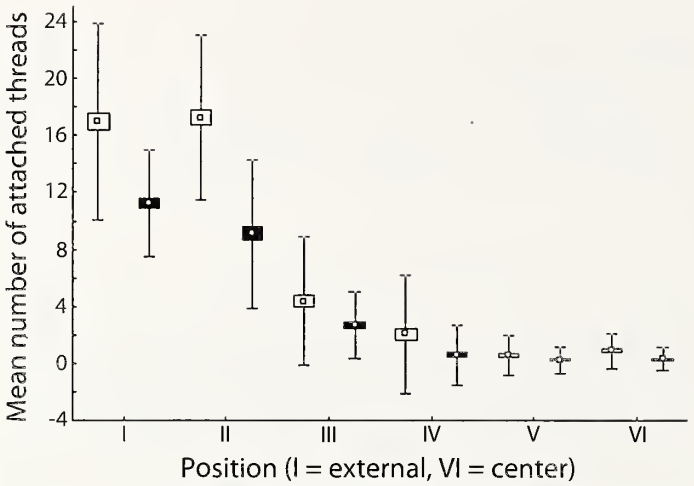

Figure 2.—Number of threads (mean, SE, and SD, $n = 32$) attached to firm strips (white boxes) and to unstable strips (black boxes), ordered from the external position (position I) to the center (position VI) (Firm substrate: B = −0.32, $R^2 = 0.82$, $F_{1,4} = 18.51$, $P < 0.0001$; Unstable substrate: B = −0.17, $R^2 = 0.70$, $F_{1,4} = 9.19$, $P < 0.0001$; comparing slopes, $t = -7.36$ $P < 0.001$). Spiders were more likely to attach threads to firm vertical surfaces. Furthermore, stable threads were more likely to be located in high and low positions, while unstable threads were more evenly distributed.

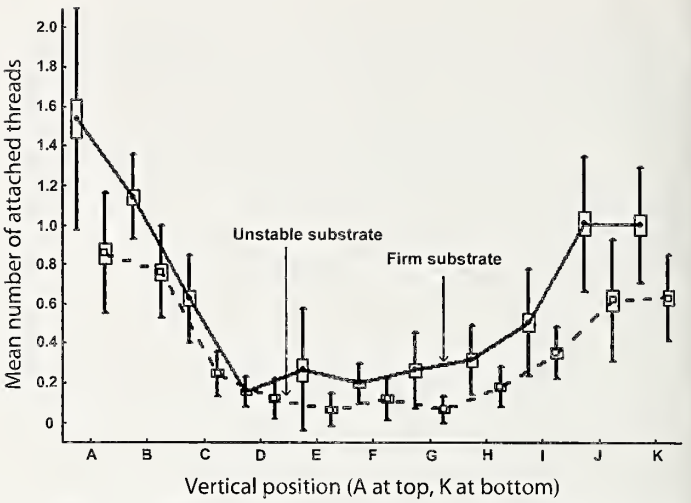

Figure 3.—Number of threads (mean, SE, SD) attached to the different vertical sections of the firm strips (solid line) and unstable strips (dotted line). Position A is at the top of the strip and position K at the bottom.

orb was not rebuilt (R. Madrigal Brenes pers. observ.). I repeated this procedure two more times and counted the threads of each of the three webs built by each spider. Webs were rebuilt within the next three nights in all cases.

To determine if spiders preferred to attach threads to the firm or the unstable strips, I first averaged for each spider the values corresponding to the three webs. I then compared the number of threads attached to pairs of strips at comparable positions in the cage (distances from the corners) using a paired t-test. I thus compared band 1 (firm) with band 12 (unstable); 2 (unstable) with band 11 (firm) (Fig. 1), and so on. To measure the distribution of threads relative to the position of the strips (horizontal axis, position 1 closest to the corner), I performed a regression for the firm strips and another one for the unstable strips and then compared the slopes to determine if the number of threads changed relative to the position of the strips in firm vs. unstable strips.

Lastly, for both firm and unstable substrates, I determined if the spider attached different numbers of threads at different heights along the length of each strip, using a Kolmogorov-Smirnov test for each type of substrate. For this test, I averaged the number of threads attached to unstable and firm substrates at each height (sections from A to K) for each of the three webs of the spiders.

All spiders ($n = 32$) attached more threads to the firm strips than to the unstable strips (mean ± SD: firm = 7.02 ± 1.15, unstable = 4.04 ± 0.85; paired $t_{31} = 18.96$, $P < 0.0001$). However, spiders did not distribute their threads evenly along either the horizontal or vertical axes. Horizontally, all spiders attached more threads to the strips nearer the corners of the cage, regardless of whether the strip was firm or unstable (Fig. 2). Along the vertical axis, spiders attached higher numbers of threads to the upper portion of each strip, followed by intermediate numbers in the lowest portions, with the fewest threads attached to positions near the center of the strip (Kolmogorov-Smirnov test, $D = 0.36$, $n = 11$, $P < 0.025$; Fig. 3).

In this experiment *Cyrtophora citricola* showed a strong preference to attach silk threads to firm substrates. The ability to select firm substrates for web construction is probably important for this species, which builds long-lasting webs. The additional preference for attaching threads near the corners rather than to the central section of the wall may be a consequence of the characteristics and shape of the orbs of this species. Orb-web building spiders tend to build long bridging lines that form part of the upper frame and support the rest of the orb web. In general, the attachment points of the anchor lines that support the frame are relatively few and tend to be well separated (Foelix 2011). Although construction behavior is yet unexplored in *C. citricola*, it is possible that this species follows a similar pattern of behavior: first build anchor and/or frame lines that are attached to extremes, and then use these lines as mechanical support to construct the rest of the web. This possibility remains to be demonstrated.

Based on an experimental approach, the results of this investigation demonstrated that *C. citricola* clearly selects firm over unstable substrates to construct its web. A similar approach may be used to test whether other spiders that construct durable webs such as species in the *Mecynogea* genus and Uloboridae family have a similar pattern of substrate selection, and to test whether spiders that construct less resistant and durable webs (e.g., *Leucauge* spp.) have a lower propensity to select firm substrates.

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SHORT COMMUNICATION

Copulatory behavior of Microstigmatidae (Araneae: Mygalomorphae): a study with *Xenonemesia platensis* from Argentina

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Abstract. Microstigmatidae are small ground-dwelling and free-living spiders. The present study reports on the copulatory behavior of *Xenonemesia platensis* Goloboff 1989, constituting the first report on sexual behavior of the Microstigmatidae. Our findings in *X. platensis* did not show evidence of pheromones associated with silk. The courtship behavioral units of males was comprised of quivers by legs I and II, brusque movements of the palps, and leg tapping with legs II. During mating, a novel courtship behavior by males was observed that consisted of tapping and scraping with legs II on the female legs. The present study not only gives a description of mating behavior in Microstigmatidae for the first time, but also reports strong evidence of nongenital copulatory courtship activity in mygalomorph spiders.

Keywords: Argentinean spider, South America, courtship, mating, reproductive biology

Many spider species could be compelling targets for evolutionary studies due to their unusual reproductive biology (Eberhard 2004); it appears that a species of microstigmatids provides just such a target. Microstigmatidae are small ground-dwelling and free-living spiders (Griswold 1985) restricted to habitats offering constant high humidity and even temperature (Lawrence 1953). This family comprises 15 species, nine of them distributed in the New World (Platnick 2011). Members of this family are characterized by rounded book-lung openings and extremely shortened posterior lateral spinnerets (Goloboff 1995). Microstigmatid species, in particular, have long been overlooked, both because of their rarity in collections and their extremely small size (adult males are 1–3 mm in total length) (Raven & Platnick 1981). The spiders are not known to construct burrows or retreats and are supposed to make minimal use of silk. They readily attack and feed upon small insects (Griswold 1985). There are few published records of either the natural history or the ecology of microstigmatid species (Griswold 1985; Dippenaar-Schoeman et al. 2006: Old World species; Indicatti et al. 2008: Brazilian species; Ferretti et al. 2010: Argentinean and Uruguayan species).

Here we report on the copulatory behavior of *Xenonemesia platensis* Goloboff 1989, constituting the first report on sexual behavior of a microstigmatid. We collected three adult males and three adult females at Martín García Island, Buenos Aires, Argentina (34°11′25″S, 58°15′38″W), in August 2009. Voucher specimens are still alive and will be deposited in the Museo de La Plata, Division Entomología, La Plata, Buenos Aires, Argentina. All the females molted before we made observations, so they did not have stored sperm. In the laboratory we kept them individually in plastic Petri dishes (9 cm diameter × 1.5 cm high), with soil as substrate and wet cotton wool moistened daily. These containers allowed us to follow their behavior as they constructed their burrows. We fed all individuals weekly with cockroaches (*Blattella germanica*) of approximately 10 mm length. We used a 12 h light/dark cycle, and the room temperature during breeding and observations was 26.7°C ± 1.52 SD.

In order to observe mating, we placed each female dish inside a larger glass cylindrical container (19 cm diameter and 10 cm high) with a layer of soil approximately 6 cm deep. A depression excavated in the center of the larger container for the female's Petri dish avoided the destruction of the female's shelter during the transfer. The mating arena was illuminated with artificial fluorescent light. For each encounter, we removed the male from his Petri dish and carefully introduced him into the larger container housing the female's dish, and at quite a distance from the female.

We performed nine male-female pairings of *X. platensis* in all combinations, and both males and females were given three possible mating opportunities. We considered only the first pairing for description of behavioral units during courtship and mating sequences because female behavior in particular may change after a first successful insemination, and since these spiders are very rare, they probably never encounter potential mates at such high frequencies. We recorded copulations with a Handycam Panasonic SDR-S7 and analyzed the video records with a PC program (Sony Vegas 9.0) in order to describe behavioral patterns accurately. We used slow motion and single frame advance modes. Durations and frequencies are given as averages ± standard deviations.

We present the frequency and duration of behavioral units during the three mating exposures and five copulations in Table 1. When *X. platensis* engaged in courtship and mated, a common pattern occurred (Fig. 1a). All males began the courtship when they directly contacted the female's body. During this initial contact, females remained largely motionless. The male did not start courtship when he contacted female silk, but did so only after contacting the female herself. Early studies proposed that mygalomorph spiders lacked chemical cues in sexual communication (Baerg 1958; Platnick 1971). However, more recent studies have reported the presence of pheromones associated with female silk threads (Costa & Pérez-Miles 2002; Ferretti & Ferrero 2008). Our findings in *X. platensis* could indicate the absence of pheromones associated with silk, but obviously more detailed studies are needed to confirm this.

After initial contact, the male quivered with the first and second pair of legs, followed by fast upward and downward movement of the pedipalps. The male made nine behavioral bouts with an average duration of 0.52s ± 0.06 SD (range = 0.44−0.60). At first glance, the quivers observed in the courtship of *X. platensis* could be similar to the body vibrations observed in some theraphosids (Costa & Pérez-Miles 2002; Ferretti & Ferrero 2008), but in *X. platensis* the quiver is generated by the first and second pair of legs instead of pair III as observed in theraphosids. After approximately 46 s, the female raised her body up to an angle of almost 60° relative to the substrate, with the first pair of legs elevated and legs III and IV over the substrate. At

Table 1.—Frequencies and durations of behavioral units during the three mating exposures of *X. platensis*. M = male, F = female, N = number, D = duration. Mean values ± SD are presented. No matings occurred in the third set of pairings, thus producing no additional observations.

	First mating						Second mating					
	Pair 1 (M1 - F1)		Pair 2 (M2 - F2)		Pair 3 (M3 - F3)		Pair 1 (M1 - F2)		Pair 2 (M2 - F3)		Pair 3 (M3 - F1)	
Behavioral units	N	D	N	D	N	D	N	D	N	D	N	D
Courtship phase												
Quivering	9	0.52s ± 0.06	21	0.53s ± 0.03	9	0.56s ± 0.08	24	0.53s ± 0.05	39	0.54s ± 0.04		
Palpal boxing	6	1.92s ± 0.95	11	2.27s ± 0.99	3	5.13s ± 3.17	5	5.34s ± 0.90	4	3.06s ± 1.04		
Leg tapping	7	1.00s ± 0.39	7	1.02s ± 0.47	4	2.46s ± 1.69	11	0.90s ± 0.36	14	1.63s ± 1.42		
Mating phase												no mating
Palpal insertions	3	25.25s ± 12.97	15	14.91 ± 10.37	6	14.13 ± 7.42	4	14.36 ± 8.70	5	18.24 ± 13.30		
Leg beating	7	9.40s ± 5.03	2	5.08s ± 1.01	7	8.48s ± 3.54	2	11.36s ± 2.03	6	8.53s ± 3.49		
Mating		4.61min		4.78min		2.73 min		1.98 min		2.01 min		

this instance, the male made alternating movements of the pedipalps, touching the genital zone of the female (palpal boxing). We usually observed palpal boxing alternated with quivers. Palpal boxing occurred six times, with an average duration of 1.92s ± 0.95 SD (range = 0.96 − 3.40 s).

Subsequently, the male vigorously hit legs I and II of the female with the tarsi of his legs II extended. This behavior consisted of high-frequency leg tapping in an alternating or synchronous phase. The male made seven leg tappings with a mean duration of 1.00 s ± 0.39 SD (range = 0.68 − 1.80). The brusque movements of the palps and the scraping with legs II during courtship have not been reported in any other mygalomorph spider. These abrupt palpal movements could be similar to the "twitching" observed in a diplurid (Coyle & O'Shields 1990), which consisted of distinct, sudden flexions or extensions of one or more legs or palps. Next, the male clasped the female's palps and chelicerae between his first pair of legs (Fig. 1b). The distal portion of each male tibia without tibial apophyses or megaspines was placed against the prolateral surface of each female pedipalp base. The male placed his second pair of legs against the female's first pair of legs, as if pushing them, and then palpal insertion attempts began.

From the nine encounters, we obtained five successful matings. All of the first copulations were successful during the first three pairings. In the second pairings, we observed two successful copulations, and no matings occurred in the third set of pairings. In one case, the female rejected the male with vigorous lateral abdominal oscillations while raising her body. In three cases, males never initiated courtship. During the copulation, the male positioned himself under the female, facing her sternum. The female's pedicel was flexed upwards so that the cephalothorax-abdomen angle was 30–50°. This mating position continued during the palpal insertion attempts, and the copulation lasted 4.61min. The male made three palpal insertions with a mean duration of 25.25s ± 12.97 SD (range = 11.96−37.88). During palpal insertion attempts, the male continued performing tapping with legs II and quivering.

Afterward, while the male was inserting his palp into the female's genital opening, he added a new behavioral unit. He raised the second pair of legs to an angle of 90° between the femur and patella and quickly moved the legs upward and downward. Male tibia, metatarsi, and tarsi remained extended, and the tarsi beat and scraped the second and third female coxae. The male performed seven repetitions of this leg-beating behavior with a mean duration of 9.40 s ± 5.03 SD (range = 4.36 − 18.88) and a velocity of 14 beats per second. The male's tapping with his second legs during copula could be interpreted as courtship in copula. This behavior, as far as we know, is unique to *X. platensis* and has not been previously reported in mygalomorphs (Costa & Pérez-Miles 1998, 2002; Ferretti & Ferrero 2008; Jackson & Pollard 1990). Finally, when the spiders separated, the male quickly moved backwards. In the observed matings of *X. platensis*, the copulation position achieved was similar to that of most mygalomorphs (Costa & Pérez-Miles 2002), and the behavior displayed by this species during mating is noticeable and unusual among mygalomorph species.

The female's apparent unresponsiveness throughout courtship and copulation may be a test of the male's quality (Eberhard 1985); she may be monitoring his overall performance, not only genital stimulation. The sexual selection by female choice hypothesis predicts selective cooperation in which males perform luring behavior, and females choose a mate according to the male's courtship display (Thornhill 1983; Eberhard 1985, 1996, 1997). One way a male may prevail in this competition is by courting the female during copulation (copulatory courtship) (Eberhard 1994, 1996) and thereby inducing her to use his sperm. Males of hundreds of species of animals perform nongenital behavior, during copulation, that appears to be courtship; this behavior includes biting, tapping, rubbing, squeezing, shaking, vibrating, singing to, and feeding the female (Eberhard 1994, 1996).

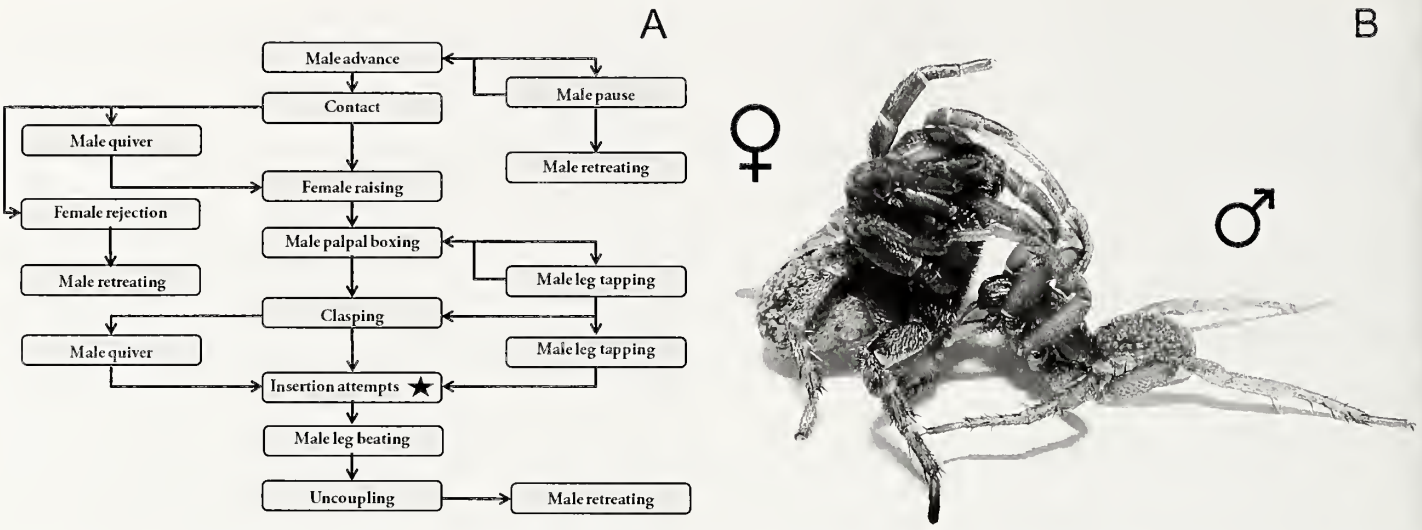

Figure 1.—Courtship and mating of *X. platensis*. a) Ethogram showing the courtship and mating pattern; the black star indicates the instance where photo was taken for Figure 1b. b) Male clasping and tapping female with forelegs during insertion attempts.

Few studies have directly tested the possibility that copulatory courtship affects paternity. In insects, copulatory courtship can result in a decrease in female mobility during copulation (Humphries 1967) and increased resistance to subsequent matings (King & Fischer 2005). These effects could be operating in the mating behavior of *X. platensis*, given the female's largely motionless state during courtship, copulation and post-copulation. They could also lead to some kind of resistance to subsequent mating, given that the three females accepted a first male, two females accepted a second male, and none accepted a third male. Obviously, this work constitutes preliminary observations, and more data are needed to elucidate these hypotheses.

In conclusion, the present study not only gives a descriptive overview of the mating behavior in the Microstigmatidae for the first time, but also reports strong evidence of nongenital copulatory courtship in mygalomorph spiders, both of which offer a promising field of research in the context of sexual selection.

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SHORT COMMUNICATION

Gregarious behavior of two species of Neotropical harvestmen (Arachnida: Opiliones: Gonyleptidae)

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Abstract. We present the first record and description of the gregarious behavior of the Neotropical harvestmen *Serracutisoma proximum* (Mello-Leitão 1922) and *Serracutisoma spelaeum* (Mello-Leitão 1933) (Opiliones: Gonyleptidae: Goniosomatinae) (DaSilva & Gnaspini 2010). We followed and described the pattern of these aggregations over a period of 17 months in a cave in southeastern Brazil. Individuals of the two species aggregated with both conspecifics and heterospecifics during the non-reproductive season (i.e., from October to March, the cool and dry season). Aggregations contained up to 81 individuals, usually with a female-biased adult sex ratio. Multispecific aggregations were usually composed mainly of representatives of one of the two species, suggesting that although these species also aggregate with heterospecifics, there is a preference for aggregating with conspecifics. This study provides novel information on the social behavior of harvestmen, specifically regarding the composition of multispecific aggregations.

Keywords: Aggregations, Goniosomatinae, *Serracutisoma proximum*, *Serracutisoma spelaeum*, social behavior

The habit of aggregating with conspecifics has been observed in several animal taxa. The forces driving this gregariousness in different organisms include defense against predators (Uetz et al. 2002), food acquisition, reproduction, selection of microhabitats, and thermoregulation (Krause & Ruxton 2002). Life in groups might present costs such as higher competition for resources, increased transmission of parasites, and easier detection by predators, but it also offers some exclusive benefits (see review by Krause & Ruxton 2002; Uetz et al. 2002).

Aggregations are common among harvestmen, having been observed in 41 species belonging to 10 families (Machado & Macías-Ordoñez 2007). In contrast to the spherical aggregations of the Old World harvestmen (Holmberg et al. 1984), Neotropical harvestmen aggregations are defined as groups of three or more individuals with bodies 0–5 cm apart from each other and legs overlapping (cf. Machado et al. 2000). Several hypotheses have been proposed to explain how and why harvestmen aggregate, including defense against predators, physiological protection against harsh winter conditions (unlikely to apply for the loose aggregations of Neotropical harvestmen; see Holmberg et al. 1984; Santos 2007), preferential selection of sites with favorable microclimatic conditions, and preferential attachment of individuals (review in Machado & Macías-Ordoñez 2007).

The harvestmen *Serracutisoma spelaeum* (Mello-Leitão 1922) and *Serracutisoma proximum* (Mello-Leitão 1933) (Laniatores: Gonyleptidae: Goniosomatinae) (DaSilva & Gnaspini 2010) are found in the Ribeira Valley, an area of Atlantic rain forest in southeastern Brazil. This area's climate is characterized by two distinct seasons: a warm and wet season between November and April, and a cool and dry one between May and October (Chelini et al. 2011). *Serracutisoma spelaeum* spends its days resting in caves and its nights foraging in the forest (Santos & Gnaspini 2002; Gnaspini et al. 2003). This species reproduces inside the caves during the warm and wet season (October to March) (Gnaspini 1995). *Serracutisoma proximum* is usually found on vegetation flanking rivers, and females usually lay their eggs on the abaxial surface of leaves hanging above the river surface (Buzatto et al. 2007; but see Ramires & Giaretta 1994; Gnaspini 1996). We found populations of *S. proximum* and *S. spelaeum* cohabiting and composing multispecific aggregations in the Moquem cave (24°18my50.4″S, 48°27′18.8″W, at an elevation of approximately 750 m), situated in the Intervales State Park, southeast São Paulo State, southeastern Brazil. Although *S. proximum* shelters in cave entrances (see Chelini et al. 2011), these two species have seldom been found in the same cave (Gnaspini 1996). This is the first record of multispecific harvestmen aggregations involving Goniosomatinae species. To date, information about the gregarious behavior of Goniosomatinae harvestman is very scarce, but here we describe the aggregations of these two species.

We followed the aggregations of *S. proximum* and *S. spelaeum* of the Moquem cave over the course of 15 field trips regularly distributed between August 2004 and December 2005. During each visit to the cave, we identified all the individuals of *S. spelaeum* and/or *S. proximum* that we found as adult males, adult females, or juveniles (immatures of the 4th and 5th instar, distinguished by their size, color, and number of tarsal articles according to Gnaspini 1995) and recorded the specific composition of all aggregations. We included the individuals described as subadults by Gnaspini et al. (2004) in the "adult" count, since they are now recognized as a smaller morph of adults (e.g., DaSilva & Gnaspini 2010).

Over the course of our 15 field trips, we found a total of 49 aggregations (Fig. 1). The aggregations were markedly more common between May and late October, the period corresponding to the non-reproductive dry and cool season (Gnaspini 1996; Buzatto et al. 2007). However, females and juveniles of *S. spelaeum* also formed small aggregations in December, January, and February. Seven aggregations were composed exclusively of *S. proximum*, 28 were composed exclusively of *S. spelaeum* and 17 were multispecific aggregations containing *S. spelaeum*, *S. proximum* and occasionally *Promitobates* sp. (Gonyleptidae: Mitobatinae). In 10 out of the 17 multispecific aggregations observed, one of the goniosomatines

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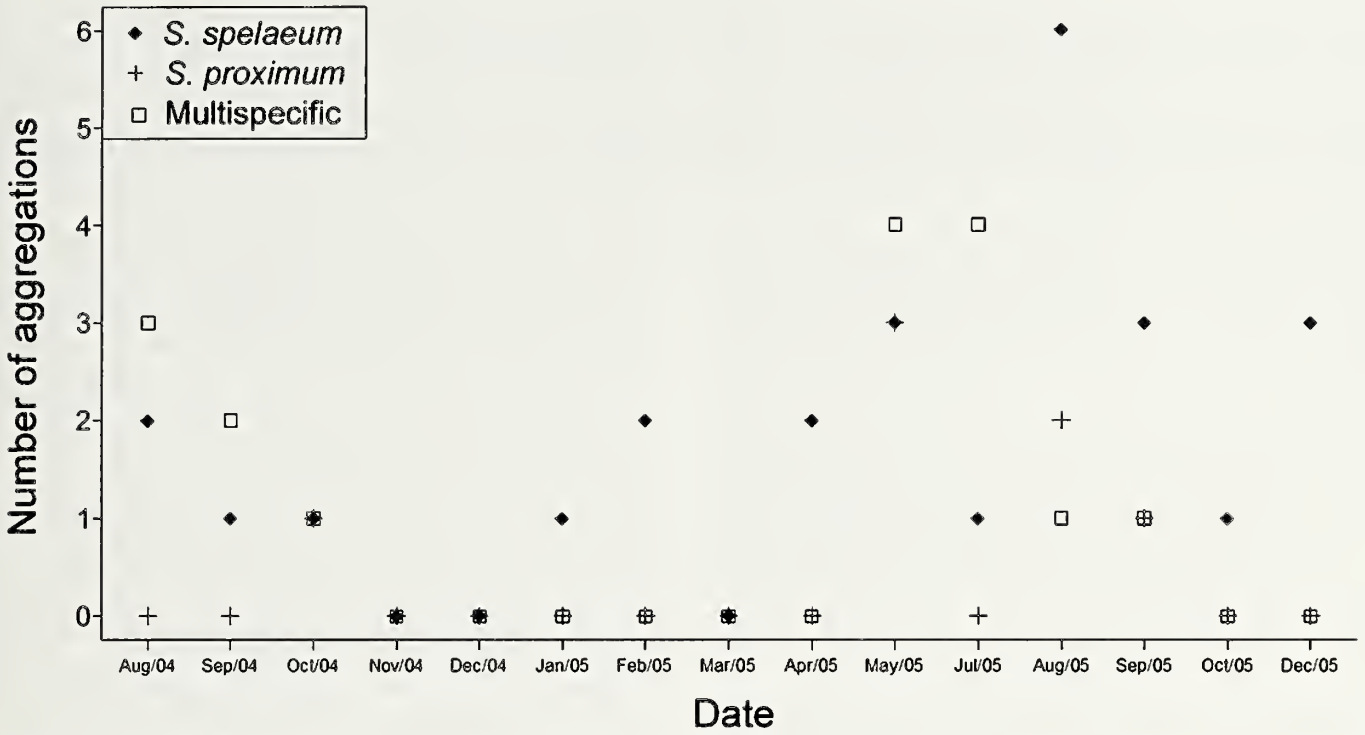

Figure 1.—Number of aggregations composed only of *Serracutisoma proximum*, *Serracutisoma spelaeum* and both species during the study period.

(either *S. proximum* or *S. spelaeum*, and more frequently the former) represented more than 75% of the total number of aggregating individuals. Juveniles of *S. spelaeum* were more common in multispecific aggregations than adults, and adults were found more frequently in conspecific aggregations.

Although the Moquem cave has several ceiling openings, exposed areas (walls directly exposed to sunlight; see Willemart & Gnaspini 2004a) represent only approximately 30% of the cave's total area. *Serracutisoma proximum* seems to aggregate more frequently on these exposed walls of the cave than *S. spelaeum* (71% of their conspecific aggregations are in exposed walls, versus 50% of the *S. spelaeum* conspecific aggregations). Some specific areas of the cave, sometimes with an area no larger than 1 m^2, seemed to be recurrently used as aggregation sites, while others were never used, suggesting that these species are highly philopatric in relation to their aggregation site (see Gnaspini 1996 and Willemart & Gnaspini 2004b for similar results on another Goniosomatinae species; see also Donaldson & Grether 2007 for an Eupnoi). Regarding the exposure to light of the aggregation sites, *Serracutisoma proximum* may remain close to the epigean habitat because it is not a typically cavernicolous speeies (Buzatto et al. 2007), while *S. spelaeum* is an obligatory cavernicolous species whose individuals prefer darker regions of the cave (Gnaspini 1996; Gnaspini et al. 2003). Supporting this hypothesis, an experimental study on the physiological preferences of several Goniosomatinae species pointed out that *S. spelaeum* constantly prefers darker refuges than *S. proximum* (Santos 2003).

The number of multispecific aggregations that we found indicates that aggregating with heterospeeifics is not just an occasional occurrence, but rather a frequent behavior exhibited by both the goniosomatines studied here. If harvestmen aggregations have a defensive function against predators, the presence of individuals from a different species could increase its defensive potential. This could be due to different sensory capabilities of a non-conspecific individual, to differences in the chemical properties of its repugnant substance, and/or simply to the increase in the number of individuals able to detect a predator (see Machado & Vasconcelos 1998). Our data suggest, however, that there is a preference for aggregating with conspecifics, independent of the aggregation placement. Gnaspini (1996) noticed that individuals of *S. spelaeum* followed only a few specific "trails" to leave from and return to the caves they used to shelter during the day. It is possible that individuals recognize conspecific chemical marks more easily, which would lead to a higher probability of finding and aggregating with conspecifics (see Donaldson & Grether 2007; Grether & Donaldson 2007). This hypothesis remains to be tested with an adequate experimental approach.

The conspecific aggregations of both species had strongly female-biased sex ratios. Although this result follows the pattern found in other harvestmen (Machado et al. 2000; Machado & Raimundo 2001; Machado 2002; Willemart & Gnaspini 2004b), it appears that the entire *S. spelaeum* population goes through a change in its sex ratio during the non-reproductive season (see also Gnaspini 1995), and that this population-biased sex ratio is reflected in the aggregations. The median adult sex ratios (male:female) of the conspecific aggregations were 1:1.54 for *S. proximum* (min–max = 5:12–3:1; $n = 7$), and 1:2 for *S. spelaeum* (min–max = 0:5–1:2, $n = 28$). The median adult sex ratio (male:female) of the *S. spelaeum* population (considering both aggregated and isolated individuals) was strongly female biased in the cool and dry months (1:2.76, min–max = 1:1.06–1:7.16), but not in the warm and wet season (1:0.88, min–max = 1:0.21–1:1.64). The median adult sex ration of the *S. proximum* population (male:female) was 1:1.16 during the cool and dry season. We could not calculate a median population sex ratio for this species during the warm and wet season, since *S. proximum* seldom enters the cave in the reproductive season (Chelini et al. 2011). Since *S. proximum* and *S. spelaeum* only form aggregations during the dry and cool season (Fig. 1), the aggregations' sex ratio cannot be calculated for the warm and wet reproductive season.

We hypothesize that this female-biased sex ratio is related to the agonistic behavior of the males. Male *S. proximum* and *S. spelaeum* are known to fight with other males during the reproductive season (Buzatto et al. 2007; M.-C. Chelini unpublished data). Some males may remain intolerant of the close presence of other males even

during the non-reproductive season. These harvestmen would benefit from the defensive advantages of being aggregated (e.g., dilution effect, confusion effect: Krause & Ruxton 2002; Uetz et al. 2002) during the cold and dry season, but would separate through the reproductive season. The frequently high intraspecific predation of eggs (Buzatto et al 2007; Willemart et al. 2007; Requena et al. 2009), along with male-male competition, could counterbalance the defensive benefits of being aggregated in the warm and wet months.

With this study, we bring new information to the knowledge of harvestmen social behavior. Although purely descriptive, our data seem to indicate that the selection of aggregation sites by *S. spelaeum* and *S. proximum* is not driven by the search for ideal microclimatic conditions, since we detected both species aggregating in places as different as the entrance of the cave (exposed to the light, subject to higher temperature fluctuation and influence of the external environment) and the aphotic zone of the cave (in complete darkness, with temperature and humidity practically constant throughout the day and throughout the year: Chelini et al. 2011). Our data are thus concordant with Willemart & Gnaspini (2004b), according to whom harvestmen aggregations are a defensive strategy incompatible with reproduction. Experimental studies remain to be designed and executed in order to test the different hypotheses relative to harvestmen's gregarious behavior and, more specifically, the costs and benefits of aggregating with heterospecifics.

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SHORT COMMUNICATION

Epizoic cyanobacteria associated with a Neotropical harvestman (Opiliones: Sclerosomatidae) from Costa Rica

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Abstract. We describe multiple observations of epizoic cyanobacteria occurring on external surfaces of a species of sclerosomatid harvestman (*Prionostemma* sp.) in Costa Rica. In the field we collected four adults (three males, one female) that had green films growing upon the dorsal surfaces of the carapace and abdominal scutum. Examination by SEM revealed dense clusters of what appeared to be small prokaryotic cells (1–5 μm in diameter) covering the external surfaces of the carapace, abdominal scutum and coxae. We extracted DNA from the films of two specimens. The DNA was used as a template to amplify the intergenic spacer region (IGS) between the beta and alpha phycocyanin subunits (a signature DNA sequence, unique to cyanobacteria) by PCR. We successfully amplified an approximately 700 base pair product using DNA extracted from the film and did not obtain any product from the harvestman lacking the film. Our observation represents the second confirmed occurrence of epizoic cyanobacteria on Neotropical harvestmen. This is the first report of cyanobacteria associated with a sclerosomatid anywhere and the first known case from Central America.

Keywords: Central America, mutualism, *Prionostemma*, symbiosis

Little is known about the ecological interactions between harvestmen and other organisms (Cokendolpher & Mitov 2007). The best-characterized associations are those between adult sclerosomatid harvestmen and ectoparasitic or phoretic mites and endoparasitic gregarines (reviewed by Cokendolpher & Mitov 2007; Townsend et al. 2008). With respect to epizoic organisms, there are relatively few observations of interactions between harvestmen and cyanobacteria (Machado & Vital 2001), nonpathogenic fungi (Machado et al. 2000) or liverworts (Machado & Vital 2001). The only previous report of an association between harvestmen and cyanobacteria is for the gonyleptid harvestman *Neosadocus* sp. (Machado & Vital 2001). In this study, four individuals (total sample size = 140 individuals) were observed with epizoic filamentous cyanobacteria growing on the dorsal surfaces of the scutum. Two of these harvestmen were also hosts for epizoic liverworts (*Aphanolejeunea subdiaphana* and *Lejeunea* aff. *confuse*). After 72 h of observation, Machado and Vital (2001) noted that the epizoic organisms did not appear to affect locomotion or behavior of their hosts. They proposed the hypothesis that harvestmen with green epizoites may benefit by having better camouflage in forested habitats and thus may be better protected from predators.

From 23–24 July 2010, we collected harvestmen from the vegetation and leaf litter in the rainforest at Las Brisas Nature Reserve, Limon Province, Costa Rica (10°23′52.08″N, 83°22′34.72″W). The habitat features a mixed primary and secondary forest at an elevation of 800 m, the lower limit of cloud forest. Tree trunks and understory vegetation were constantly wet due to the high humidity, and many surfaces were covered by dense mats of epiphytic mosses. During the evening of 23 July, we collected three male and one female *Prionostemma* sp. that exhibited a bright green dorsal surface (Figs. 1, 2). In contrast to the males, the female had a darker posterior patch on the abdominal scutum (Fig. 2). Each individual was photographed, collected by hand and preserved in 70% ethanol. Within 12 hrs of immersion in ethanol, the green on the specimens had faded, although individuals still retained small irregularly spaced traces of green. We identified the source of the color as a thin film that covered the dorsal surface. After 24 h, the dorsal surfaces of the bodies of the specimens had become entirely white. In August, we carefully removed a small portion of the thin film from a single specimen and mounted the material on a glass slide. Observation with a compound microscope under oil immersion revealed that the film was made up of dense clusters of relatively small cells (1–5 μm in diameter). We prepared an adult male for examination by scanning electron microscopy (SEM). We carefully removed the legs and dehydrated the specimen in a graded ethanol series. The male was dried with hexamethyldisilizane, mounted on an aluminum stub with double-stick tape, and sputter-coated with gold. We examined and photographed the specimen with a Hitachi S-3000N SEM at an accelerating voltage of 15 kV in the Microscopy Center at the University of Louisiana at Lafayette, Louisiana, USA. Examination by SEM confirmed our observations made with light microscopy. The external surfaces of the carapace, abdominal scutum, and coxae of the adult male were covered in dense patches of small cells (Figs. 3–5). These cells varied in size from 1–5 μm in diameter (Fig. 5), but were not organized into long chains or filaments.

In February 2011, we carefully removed samples of the films from one male and the female harvestmen with fine-tipped forceps. The film tended to fragment and was collected in approximately 1 ml of 70% ethanol and placed in a 1.5 ml microcentrifuge tube. For a negative control, we removed the dorsal surface of the abdomen of a syntopic sclerosomatid harvestman lacking the film in 1 ml of ethanol and placed it in a 1.5 ml tube. Particulate matter was collected by centrifugation for five min at 10,000 × g. The ethanol was decanted and the residue dried at 37 °C for two h. DNA was extracted from the samples using a GE Healthcare Illustra Bacteria Genomic Mini Spin Kit, following the protocol for gram positive bacteria supplied with the kit. The sample was eluted from the column in 200 μl of TE buffer (10 mM Tris HCL, 1 mM EDTA, pH = 8.0). DNA obtained from this preparation was concentrated by ethanol precipitation and dissolved in 20 μl of 0.1× TE buffer, pH 8.0, and stored at 4 °C. For a positive control, genomic DNA was extracted from 1 ml of an *Oscillatoria sp.* culture (Carolina Biological Supply) using the same

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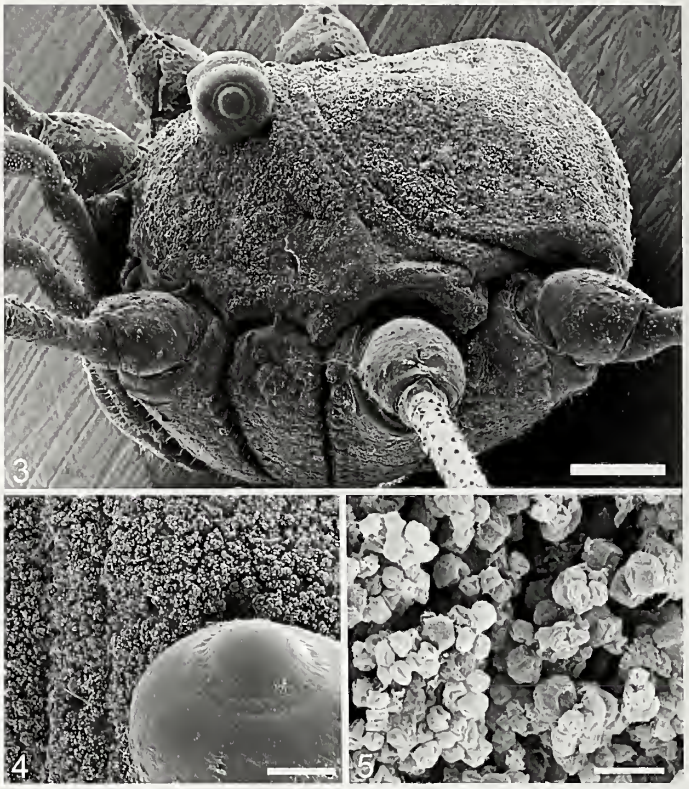
Figures 1, 2.—Photographs of living *Prionostemma* sp. with cyanobacteria on dorsum. 1. Dorsal view of adult male; 2. Dorsal view of adult female.



Figures 3–5.—Scanning electron micrographs of cyanobacteria associated with the scutum of *Prionostemma* sp. 3. Dorsolateral view of habitus showing distribution of cyanobacteria on carapace and opisthosoma; 4. Dorsal view of carapace adjacent to eye showing mass of cyanobacteria; 5. Dorsal view of cyanobacteria from abdominal scutum. Scale bars for 3 = 500 μm; 4 = 100 μm; and 5 = 10 μm.

protocol, except that the DNA eluted in 200 μl of TE was not further concentrated.

Amplification of the phycocyanin intergenic spacer (IGS) was performed using a modification of the protocol by Neilan et al. (1995). IGS forward (5′GGCTGCTTGTTTACGCGACA-3′) and IGS reverse (5′-CCAGTACCACCAGCAACTAA-3′) primers (Sigma Life Science) were used to amplify the IGS with PureTaq RTG PCR beads (GE Healthcare). Electrophoresis was carried out in 0.5 × TAE buffer, pH = 8.0, at 75 volts. The 100 BP PCR DNA Ladder (Fisher Scientific) was used to estimate fragment sizes. Gels were photographed using a Fotodyne ultraviolet light box equipped with a camera hood and filters for visualizing ethidium bromide-stained DNA. Gel images were captured with a Nikon Coolpix 4500 digital camera.

The phycocyanin intergenic spacer is a reliable marker routinely used for detecting the presenee of diverse cyanobacterial species (Neiland et al. 1995; Baker et al. 2001; Kumari et al. 2009). The forward and reverse primers were chosen to be homologous with completely conserved regions within the beta (forward primer) and alpha (reverse primer) subunits of the phycocyanin peptide sequence (Neiland et al. 1995). The length of the intergenic spacer is known to vary among cyanobacterial species; with these primers a product of approximately 700 bp is amplified from the majority of species (Neiland et al. 1995). For example, using the published sequence of *Oscillatoria* sp. PCC 6506 (accession # CACA01000001.1), a product of 691 bp is predicted. This product was obtained from the positive control (Fig. 6, lane E). A similarly sized product was amplified from genomic DNA extracted from the film recovered from the dorsal surface of both specimens (Fig. 6, lanes B and C), while no product was obtained from the harvestman lacking the film that we used as a negative control (Fig. 6, lane F).

Our observations of epizoic cyanobacteria on the sclerosomatid harvestmen from Costa Rica represent the second report of this interaction in the Neotropics and the first observation from Central America. In contrast to the 3% association rate reported by Machado & Vital (2001), we observed cyanobacteria on the dorsa of all four individuals of the species that were collected. This species, however, was not the only harvestman at the field site. In the rainforests at Las Brisas Nature Reserve, we captured adults of three syntopic sclerosomatid species as well as individuals of 14 other species, including representatives of the Cosmetidae, Gonyleptidae, Manaosbiidae, Stygnommatidae, and Zalmoxidae. No individuals of these species were green. In contrast to the filamentous cyanobacteria reported by Machado & Vital (2001), the cyanobacteria that we observed grew in a thin film. We infer from this difference in growth patterns that the harvestmen from Costa Rica interact with a different type of epizoic cyanobacteria than those in Brazil.

In general, our field observations support the Machado & Vital (2001) hypothesis that a mutualistic association exists between epizoic cyanobacteria and harvestmen. We found that the selerosomatid harvestmen with the epizoic cyanobacteria were challenging to capture because their green bodies and dark legs made them difficult to locate among the moss-covered surfaces of tree trunks in the rainforest. Thus, we infer that cyanobacteria confer some benefit to the harvestman through enhanced camouflage or crypsis. If the relationship is mutualistic, however, additional field or laboratory studies are needed to assess how the cyanobacteria benefit from this interaction. Martínez-Torrez et al. (2011) hypothesized that the cuticles of diplopods provide more stable substrates for epizoic bryophytes to survive and disperse than the surrounding litter or soil. Lücking et al. (2010) suggested that epizoic liverworts and lichens on

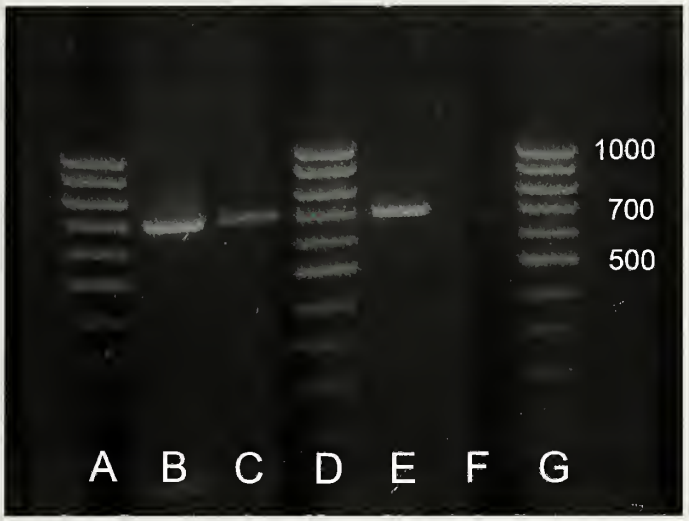


Figure 6.—Gel electrophoresis of the PCR products obtained using primers specific for the IGS sequence of the phycocyanin gene. Lanes A, D, and G: 100 BP PCR DNA ladder. Lane B: successful amplification of the IGS sequence (approximately 700 bases) from the film sample removed from the male. Lane C: successful amplification of the IGS sequence from the film sample removed from the female. Lane E: successful amplifieation of the IGS sequence from the cyanobacteria *Oscillatoria* (positive control). Lane F: PCR results for syntopic *Prionostemma* specimen without film (negative control), with no visible product obtained.

the shield mantis, *Choeradodis*, were opportunistic colonizers, taking advantage of the surface properties of the integument and the relatively long lifespan of the host. Epizoic cyanobacteria on harvestmen may also simply be taking advantage of favorable surface conditions for growth. Interestingly, most species of harvestmen are nocturnal (Goodnight & Goodnight 1976; Acosta et al. 1995; Gnaspini 1996; Machado et al. 2000; Willemart & Gnaspini 2004; Donaldson & Grether 2007; Grether & Donaldson 2007). During the day adult sclerosomatid harvestmen may gather in loose or dense aggregations on the shaded surfaces of palms or hardwoods or hide in recesses of tree buttresses or under the cover of logs or rocks (Donaldson & Grether 2007; Grether & Donaldson 2007). This pattern of behavior would seem to impact negatively epizoic organisms that require light for photosynthesis. However, if having a green dorsum provides better camouflage and enables harvestmen to be active during the day (i.e., increasing opportunities for foraging and providing more energy for growth and reproduction), then a mutualistic relationship may eonfer additional competitive advantages to both host and epizoite.

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Use only the metric system unless quoting text or referencing collection data. If English measurements are used when referencing collection data, then metric equivalents should also be included parenthetically. All decimal fractions are indicated by a period (e.g., −0.123). Include geographic coordinates for collecting locales if possible, using one of the following formats: 0°12′32″S, 29°52′17″E or 0.2089°S, 29.8714°E.

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Citation of taxa in the text: Include the complete taxonomic citation (author & year) for each arachnid taxon when it first appears in the abstract and text proper. For Araneae, this information can be found online at http://research.amnh.org/iz/spiders/catalog/. For example, *Aranens diadematus* Clerck 1757. Citations for scorpions can be found in the *Catalog of the Scorpions of the World (1758–1998)* by V. Fet, W.D. Sissom, G. Lowe & M.E. Braunwalder. Citations for pseudoscorpions can be found at http://www.museum.wa.gov.au/catalogues/pseudoscorpions/. Citations for some species of Opiliones can be found in the *Annotated Catalogue of the Laniatores of the New World (Arachnida, Opiliones)* by A.B. Kury. Citations for other arachnid orders can be found in *Catalogne of the Smaller Arachnid Orders of the World* by M.S. Harvey.

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Carico, J.E. 1993. Trechaleidae: a "new" American spider family. Pp. 305. *In* Proceedings of the Ninth International Congress of Arachnology, Panama 1983. (W.G. Eberhard, Y.D. Lubin & B.C. Robinson, eds.). Smithsonian Institution Press, Washington, D.C.

Huber, B.A. & W.G. Eberhard. 1997. Courtship, copulation, and genital mechanics in *Physocyclus globosus* (Araneae, Pholcidae). Canadian Journal of Zoology 74:905–918.

Krafft, B. 1982. The significance and complexity of communication in spiders. Pp. 15–66. *In* Spider Communication: Mechanisms and Ecological Significance. (P.N. Witt & J.S. Rovner, eds.). Princeton University Press, Princeton, New Jersey.

Platnick, N.I. 2011. The World Spider Catalog, Version 12.0. American Museum of Natural History, New York. Online at http://research.amnh.org/iz/spiders/catalog/

Roewer, C.F. 1954. Katalog der Araneae, Volume 2a. Institut Royal des Sciences Naturelles de Belgique, Bruxelles.

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